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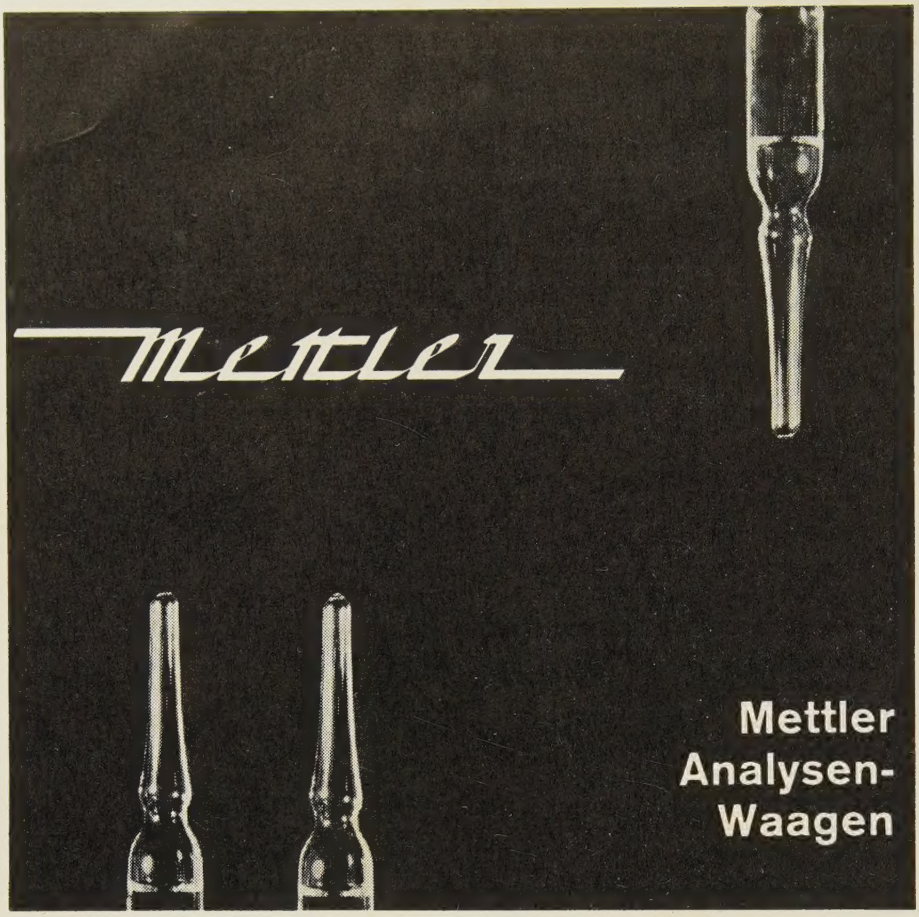

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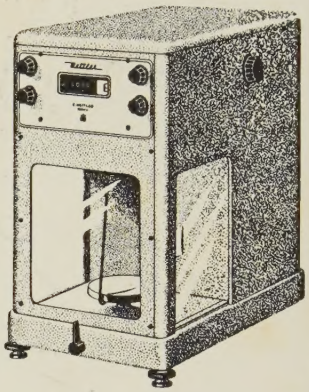

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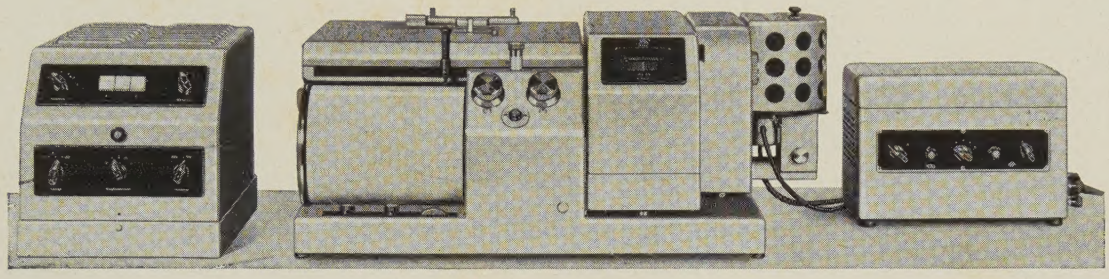

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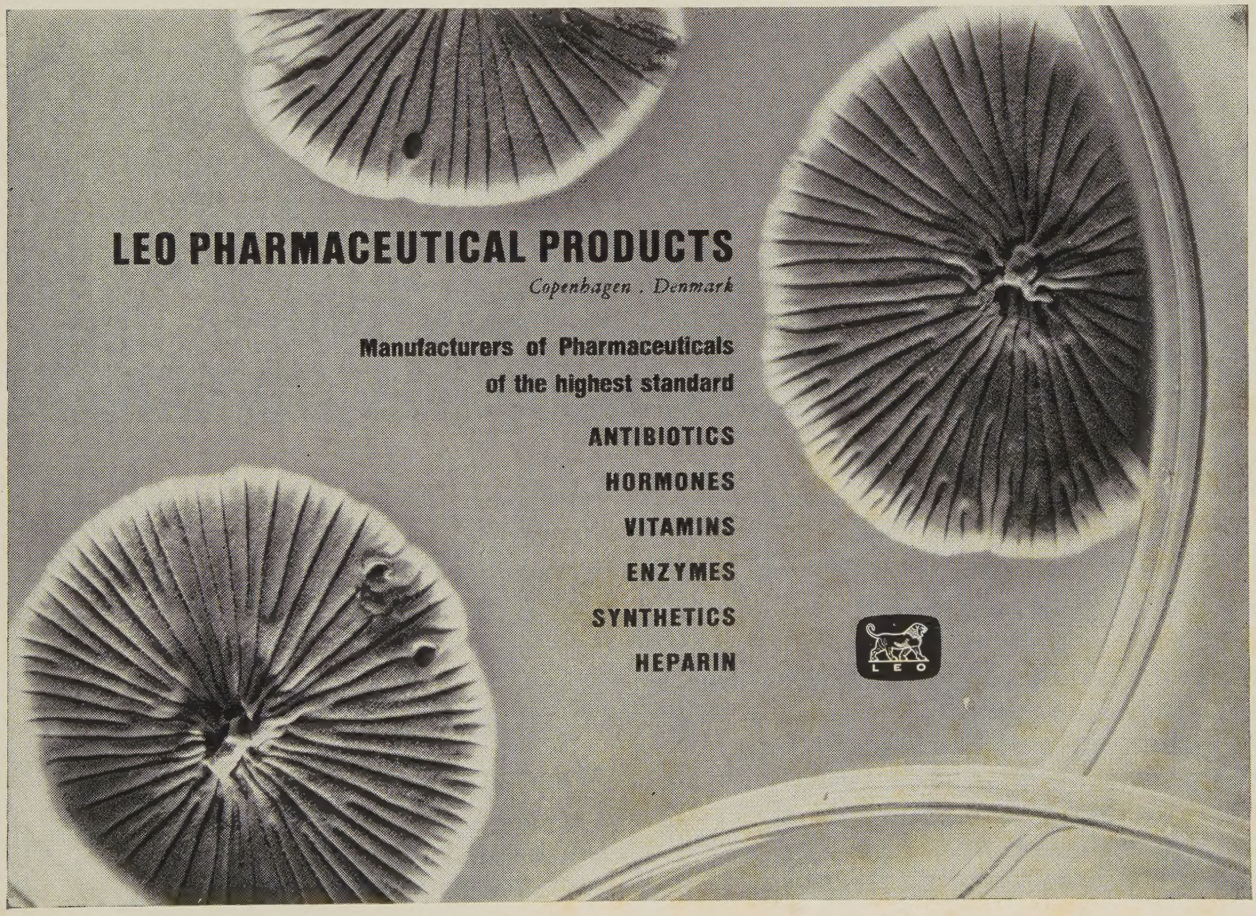

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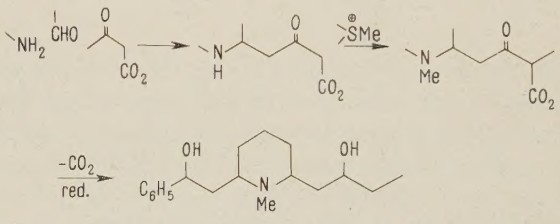
Alkaloid Biosynthesis*

By ERNEST WENKERT**

The last decade has witnessed rapid progress in the chemist's understanding of Nature's methods of synthesis of whole classes of naturally occurring substances. The acetate-polyacetate pathway, long accepted as the route to lipids[1], has been established as the basis of the biosynthesis of natural phenolic-enolic compounds[2] and has been suggested as that of macrolide antibiotics[2–4]. A slight variant, the acetate-mevalonate path, constitutes the acknowledged route to terpenes and steroids[5]. In contrast to these dramatic developments only little headway has been made in our knowledge of the biosynthesis of alkaloids[6].

The determination of the structures of alkaloids and their synthesis for the last forty years have led to the build-up of a vast body of biosynthetic theory whose main common thesis is the assumed intimate relationship between alkaloid biosynthesis and amino acid or protein metabolism[7]. This postulated consanguinity has yielded the impetus for experimentation on many *in vitro* syntheses of alkaloids from amino acids or their equivalents under 'physiological conditions'[7]. It has led to C^{14} tracer experiments being carried out almost exclusively with radioactive amino acids as starting substrates[6]. For a long time it even formed the basis for theories attempting to explain the reason for the existence of alkaloids in higher plants[8]. Despite this overwhelming unanimity of opinion it has been apparent for some time that parts or the whole of the skeleta of some alkaloids cannot be derived from a biosynthetic alkaloid-amino-acid affinity. The acetate unit appears to play an important role in the formation of alkaloids. Thus it appears as such or in a masked form[9] in many systems. As acetoacetate it is responsible for the 'acetone' moiety in the following types of bases: hygrine, cuscohygrine, cocaine, isopelletierine and pseudopelletierine[7,10], for the butyryl unit in pilocarpine[7] and for the hydroxybutyl sidechain in lelobanidine (I) and lobinine:



I

Three acetates appear responsible for the resorcinol unit in the acridone alkaloids[2,7] as well as C-2 and the *n*-amyl sidechain in the quinoline alkaloids of the Angostura bark[11]. Finally, the presence of isoprene units in many furoquinoline systems[7], in some tropane bases[12] and in a few isoquinoline alkaloids[13] as well as

* From a lecture, entitled *Alkaloid Biosynthesis*, presented on October 8, 1958, as one of a series of ten lectures on *Recent Advances in the Chemistry of Natural Products* sponsored by the New York Section of the American Chemical Society during fall of 1958.

** Department of Chemistry, Iowa State College, Ames, Iowa, U.S.A.

[1] H. J. DEUEL, Jr., *The Lipids*, Vol. III: *Biochemistry* (Interscience Publishers, Inc., New York 1957).

[2] A. J. BIRCH, *Biosynthetic Relations of Some Natural Phenolic and Enolic Compounds*, in L. ZECHMEISTER, *Progress in the Chemistry of Organic Natural Products*, Vol. XIV (Springer Verlag, Vienna, Austria 1957); A. J. BIRCH, R. A. MASSY-WESTROPP, R. W. RICKARDS, and H. Smith, J. chem. Soc. (*1958*), 360.

[3] R. B. WOODWARD, *The Structure and Biogenesis of the Macrolides, A New Class of Natural Products*, in *Festschrift Arthur Stoll* (Birkhäuser, Basel 1957).

[4] V. MUSÍLEK and V. ŠEVČIK, Naturwiss. *45*, 86 (1958).

[5] H. RILLING, T. T. TCHEN, and K. BLOCK, Proc. Nat. Acad. Sci. *44*, 167 (1958).–J. W. CORNFORTH, R. H. CORNFORTH, G. POPJÁK, and I. Y. GORE, Biochem. J. *69*, 146 (1958).–D. ARIGONI, Exper. *14*, 153 (1958).–A. J. BIRCH, R. W. RICKARDS, and H. SMITH, Proc. chem. Soc. *1958*, 192.–A. J. BIRCH, R. W. RICKARDS, H. SMITH, A. HARRIS, and W. B. WHALLEY, Proc. chem. Soc. *1958*, 223.–J. J. BRITT and D. ARIGONI, Proc. chem. Soc. *1958*, 224.

[6] L. MARION, Bull. Soc. chim. France *1958*, 109.

[7] *Cf.* R. ROBINSON, *The Structural Relations of Natural Products* (Clarendon Press, Oxford, England 1955).

[8] *Cf.* K. MOTHES, *Über die Stellung der Alkaloide im Gesamtstoffwechsel*, in *Festschrift Arthur Stoll* (Birkhäuser, Basel 1957).

[9] *Cf.* R. B. WOODWARD, Nature *162*, 155 (1948).

[10] The derivation of the 'acetone' moiety from acetonedicarboxylic acid[7] is most unlikely because of the absence of dicarboxy alkaloid derivatives in nature and, more importantly, because of the unavailability of any realistic biochemical pathway leading to dicarboxyacetone (*cf.*: A. J. BIRCH, *Biosynthetic Theories*, in A. TODD, *Perspectives of Organic Chemistry* (Interscience Publishers, Inc., New York 1956)).

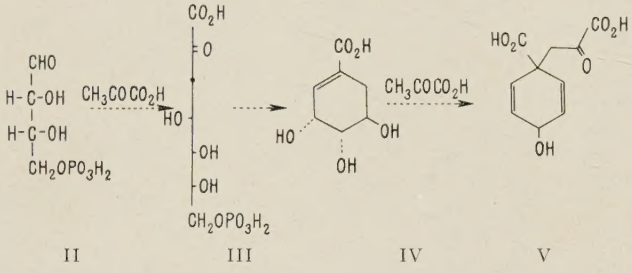
[11] C. SCHÖPF and G. LEHMANN, Ann. Chim. *497*, 7 (1932).–C. SCHÖPF and K. THIERFELDER, Ann. Chim. *518*, 127 (1935).

[12] W. DECKERS and J. MAIER, Chem. Ber. *86*, 1423 (1953).–J. B. JONES and A. R. PINDER, Chem. & Ind. *1958*, 1000.

[13] C. DJERASSI, S. K. FIGDOR, J. M. BOBBITT, and F. X. MARKLEY, J. Amer. chem. Soc. *79*, 2203 (1957).–C. DJERASSI, T. NAKANO, and J. M. BOBBITT, Tetrahedron *2*, 58 (1958).

the existence of monoterpene[14], diterpene and steroid alkaloids illustrate further utilization of the acetate building block.

Two recent discoveries have yielded clues regarding the biosynthetic origin of the majority of alkaloids, those not derivable from acetate units. Firstly, the biosyntheses of the non-resorcinol-phloroglucinol aromatic nucleus[15] and the indole ring[16] have been clarified and, secondly, this disclosure has been utilized successfully in the interpretation of the absolute configuration of the yohimbé, strychnos and cinchona alkaloids[17]. The brilliant work by DAVIS[15] and by SPRINSON[15] has shown that such aromatic systems as anthranillic and oxygenated benzoic acids, phenylalanine and tyrosine are synthesized in bacterial cells from carbohydrates via D-erythrose-4-phosphate (II), 2-keto-3-desoxy-7-phospho-D-glucoheptonic acid (III), shikimic acid (IV) and prephenic acid (V):



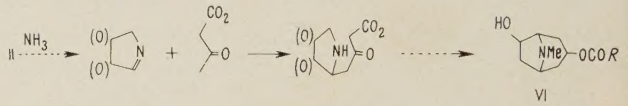
II III IV V

The revelation of this biosynthetic route was followed rapidly by suggestions of its possible universality. Thus the shikimate-prephenate pathway could account for the origin of major structural features of depsides and the tannins[18], flavonoid plant constituents[18, 19] and was proved experimentally to be responsible for the C_6-C_3 units of lignin[20].

The surprisingly wide distribution of C_6–C_1, C_6-C_2 and C_6–C_3 units among alkaloid structures, if not as part of the alkamine moieties then as the prosthetic groups, suggests strongly that even in this vast realm of naturally occuring substances the predominant route of biosynthesis is the shikimate-prephenate pathway. It is the purpose of the ensuing discussion to illustrate this relationship between alkaloid biosynthesis and carbohydrate metabolism in light of present-day knowledge in biochemistry. As will become apparent, all alkaloids are derivable, at least formally, from formaldehyde, acetate, pyruvate, erythrose, shikimate and prephenate units by routes which are analoguous in every detail to well-known enzymic reactions.

The C_4 Unit

The origin of the pyrrolidine moiety, appearing in both masked and unmasked fashion as part of the structures of many alkaloids, has been associated for a long time with the metabolism of ornithine or its equivalents[6,7]. While the biosynthetic importance of the amino acid cannot be ignored, its equivalence at this time with erythrose, an early intermediate in the shikimate-prephenate scheme, as a pyrrolidine precursor may be more than coincidence. Thus the tropane alkaloids, whose alkamines occur naturally as esters of, among others, benzoic acid, a dehydration product of shikimic acid, and tropic acid, an aldol product of formaldehyde and prephenate units, may well have their C_4-N ring originate from variously oxygenated succindialdehyde equivalents, themselves derivable from erythrose. The highly oxidized bases, e.g. teloidine (VI), are most likely to find their genesis in this pathway[21]:



VI

Similarly, in the absence of any knowledge regarding the origin of the alkaloidal nitrogen atom the carbohydrate route of biosynthesis cannot be ignored even for the hygrine, nicotine and senecio bases.

The C_4 unit appears to be an important structural moiety in alkaloids derivable from anthranillic acid (VII), itself probably produced from shikimate[15]. A combination of an aminated C_4 unit and reduced anthranillate leads to vasicine (VIII)[7,22], while a slightly varied anthranillate-erythrose complex may be

[14] K. WIESNER, Z. VALENTA, B. S. HULBERT, F. BICKELHAUPT, and L. R. FOWLER, J. Amer. chem. Soc. *80*, 1521 (1958).

[15] B. D. DAVIS, *Biosynthesis of the Aromatic Amino Acids*, and D. B. SPRINSON, *The Biosynthesis of Shikimic Acid from Labeled Carbohydrates*, in W. D. MCELROY and H. B. GLASS, *A Symposium on Amino Acid Metabolism* (The Johns Hopkins Press, Baltimore, Maryland 1955).–P. R. SRINIVASAN, M. KATAGIRI, and D. B. SPRINSON, J. Amer. chem. Soc. *77*, 4943 (1955).–E. B. KALAN, B. D. DAVIS, P. R. SRINIVASAN, and D. B. SPRINSON, J. biol. Chem. *223*, 907, 913 (1956).

[16] C. YANOFSKY, J. biol. Chem. *223*, 171 (1956), *224*, 783 (1957).–L. W. PARKS and H. C. DOUGLAS, Biochim. Biophys. Acta *23*, 207 (1957).

[17] E. WENKERT and N. V. BRINGI, J. Amer. chem. Soc. *81*, 1474 (1959).

[18] *The Chemistry of Vegetable Tannins, A Symposium*, Croydon, Society of Leather Trades' Chemists (G. Marshall and Co., Ltd. London 1956).

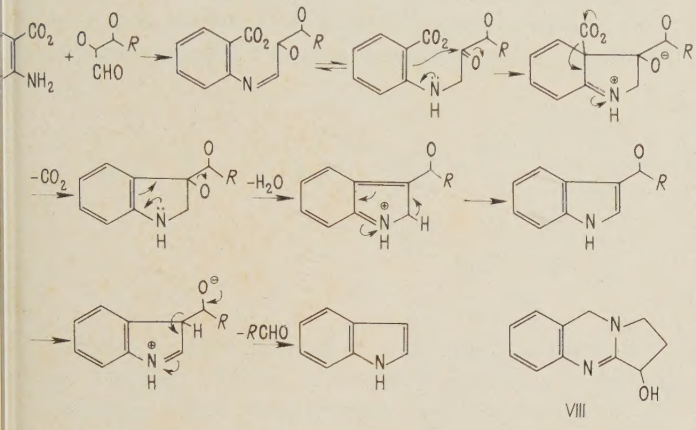
[19] E. W. UNDERHILL, J. E. WATKIN, and A. C. NEISH, Can. J. Biochem. Biophys. *35*, 219, 229 (1957).–T. A. GEISSMAN and T. SWAIN, Chem. & Ind. 984 (1957).–S. SHIBATA and M. YAMAZAKI, Chem. Pharm. Bull. *6*, 42 (1958).

[20] S. N. AZERBO, W. J. SCHUBERT, and F. F. NORD, J. Amer. chem. Soc. *80*, 1990 (1958) and preceding papers.

[21] Unfortunately former labelling experiments[6] in this field are inadequate to differentiate between the amino acid and carbohydrate routes. Contrary to claim, the *in vivo* C^{14} incorporation of atropine from labelled ornithine does not prove the latter to be a progenitor of the tropane base. It merely illustrates that the amino acid is an efficient producer of a succindialdehyde equivalent, ROBINSON's brilliantly conceived hypothetical precursor of the tropane alkaloids (R. ROBINSON, J. chem. Soc. *111*, 762 (1917)), but leaves the actual origin of the C_4 unit open to question.

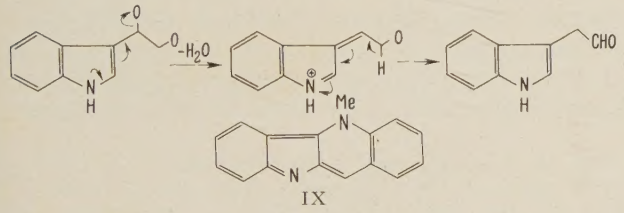
[22] C. SCHÖPF and F. OECHLER, Ann. Chim. *523*, 1 1936).

the phytochemical precursor of the indole bases coexisting with vasicine in *Peganum Harmala*. The reaction sequence responsible for the formation of the indole ring has ample analogy in the indole biosynthesis in bacteria, which recently has been shown to emanate from anthranillate and simple monosaccharides[16], and whose pathway can be portrayed mechanistically in the following manner:



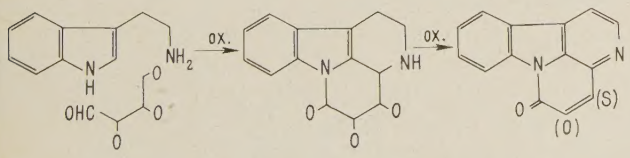
VIII

While a combination of thus-derived indole and an anthranillate unit can account for the structure of cryptolepine (IX)[7], most indole bases are substituted tryptamines or tryptophols whose origin has been considered to be tryptophan[7], itself arising from anthranillate, via indole, as above[16]. But again, the amino acid pathway is indistinguishable from its carbohydrate counterpart at this time. Thus the following dehydrative break-up of an anthranillate-erythrose complex presents a simpler, alternate route to indole alkaloids:



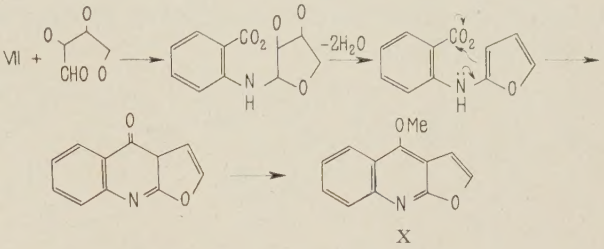
IX

The Piptadenia[23], Physostigma[7] and Calycanthus[7] bases evolve readily from the resulting aldehyde. Plants producing the Harmala and Evodia bases appear to utilize only the shikimate-prephenate scheme—the biosynthetic components being tryptamine, pyruvate, formaldehyde, and anthranillate[7]. Even the structurally unusual 'canthine' bases[7] are derivable from similar precursors, tryptamine and erythrose:



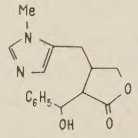
[23] M. S. FISH, N. M. JOHNSON, and E. C. HORNING, J. Amer. chem. Soc. *77*, 5892 (1955).

A slight variant of the above interactions of anthranillate and erythrose lead to the furoquinoline alkaloids, e.g. dictamnine (X):



X

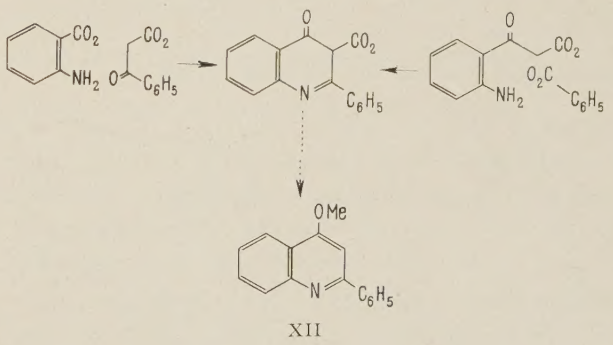
The demonstration of the derivation of the sidechain and two nuclear carbon atoms of histidine from carbohydrate sources[24] strongly suggests that imidazole alkaloids also are derived from sugars in paths parallel to the biosynthesis of histidine[7]. It is of interest that a prephenate unit, histamine and hexose moieties make up the structure of casimiroedine, a recently described imidazole alkaloid[25], while a prephenate is part of the condensed carbon skeleton in the Jaborandi alkaloid pilosine (XI).



XI

The C_6–C_1 Unit

The variously substituted benzoate group, derivable from shikimate (C_6–C_1), has been shown already to be a conspicuous part of many alkaloid structures. In most previous cases it has appeared along side C_4 or acetate units, but in many alkaloids it is revealed in conjunction with a prephenate moiety, e.g. the alkaloids of the Angostura bark and the Lunasia base, 4-methoxy-2-phenylquinoline (XII)[26]:



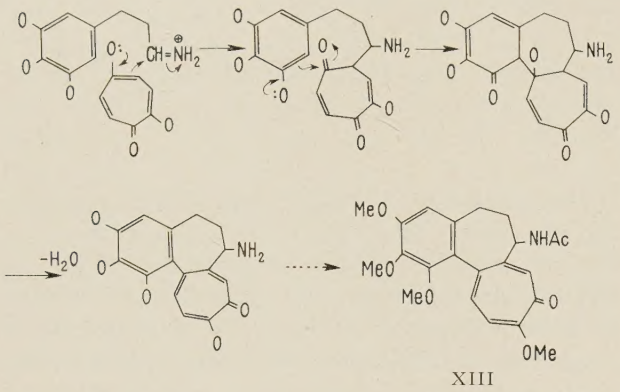
XII

[24] H. S. MOYED and B. MAGASANIK, J. Amer. chem. Soc. *79*, 4812 (1957).– B. N. AMES, J. biol. Chem. *226*, 583 (1957), and preceding papers.

[25] C. DJERASSI, C. BANKIEWICZ, A. L. KAPOOR, and B. RINIKER, Tetrahedron *2*, 168a (1958).

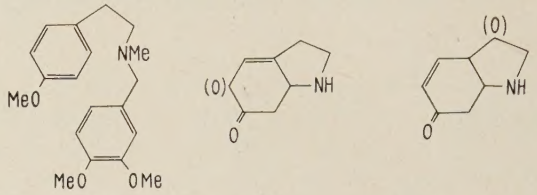
[26] S. GOODWIN, A. F. SMITH, and E. C. HORNING, J. Amer. chem. Soc. *79*, 2239 (1957).

Another shikimate-prephenate interaction is apparent from the structure of colchicine (XIII). The tropolone ring may arise either from a C_6–C_1→C_7 change or, in analogy with mold metabolites[27], directly from acyclic carbohydrate precursors[28]. The following portrays a possible biosynthetic route:



XIII

The Amaryllidaceae represent a class of alkaloids whose biosynthesis also involves the union of a C_6–C_1 entity and prephenate. In an early hypothesis the structure of lycorine (XVII), one of the most abundant bases of this class, was envisaged to be derived from the interaction of two dioxyphenylalanine equivalents and a formaldehyde unit by way of an oxidative phenyl-phenyl coupling and a retro-Michael extrusion of a twocarbon residue[29]. While this accounted for the presence of the C_6–C_1 fraction, it did not support readily the hydroaromatic state of oxidation of ring C[7]. Unfortunately this drawback also plagues a more recent scheme[30], wherein oxidative phenyl-phenyl couplings led to ketonic intermediates, which would be expected to enolize (and thus aromatize ring C) in a cellular environment. However, all difficulties are overcome, if it be assumed that hydroaromatic precursors are involved in the formation of the hydrodiphenyl system. Hence the C_6–C_1 residue corresponds to a shikimate (or IV + formaldehyde–CO_2), while the remaining C_6–C_2N moiety must emanate from prephenate. With the exception of belladine (XIV)[31], whose genesis lies in the oxidation of shikimyl 'prephenyl' amine, all Ammaryllidaceae bases most probably originate from XV and XVI, whose own biosynthesis will be discussed in the Prephenate Section.



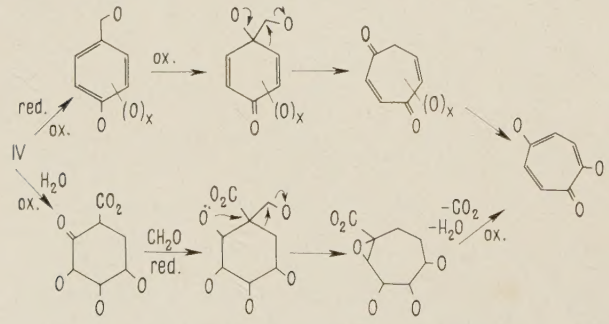
XIV XV XVI

As illustrated in Chart I, internal Michael condensation of a shikimyl derivative of XV, followed by hydration-dehydration, oxidation-reduction changes, leads to alkaloid skeleta characteristic of the lycorine group of substances, e.g. lycorine (XVII), lycorenine (XVIII)[32]. Similarly, the shikimyl derivative of XVI acts as the progenitor of the crinine group (XIX), e.g.: crinine (XXII)[33], the galanthamine variety (XX), e.g.: narwedine (XXIII)[34], and the tazettine type of alkaloids (XXI), e.g.: tazettine (XXIV)[35]. It is noteworthy that the present scheme accounts accurately for the location of oxygen atoms in the Amaryllidaceae alkaloids[36].

The tannins are a group of non-alkaloidal plant products whose structures reveal their carbohydrate-shikimate origin[18]. To account for the presence of the often recurring gallate unit, usually in oxydiphenyl or diphenyl ether form, it has been tempting to suggest

[27] R. BENTLEY, Biochim. biophys. Acta *29*,666 (1958).

[28] The route involving a transformation of shikimate to tropolone (or carboxytropolone) would require the intermediacy of shikimyl alcohol, or its equivalent, which could arise by direct phytochemical reduction of the carboxy group or by the introduction of a formaldehyde unit and the extrusion of CO_2. Furthermore, the state of oxidation of the substrate undergoing ring expansion cannot be described with certainty at this time. The following schemes illustrate two possible extreme representations of the C_6—C_1→C_7 metamorphosis:



Finally, the interaction of the C_7 unit with prephenate might occur at the tropolone or any of the pre-tropolone stages.

[29] E. WENKERT, Chem. & Ind. *1953*, 1088.

[30] D. H. R. BARTON and T. COHEN, in *Festschrift Arthur Stoll* (Birkhäuser, Basel 1957), p. 117.

[31] E. W. WARNHOFF, Chem. & Ind. *1957*, 1385.

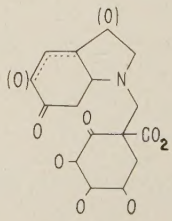
[32] T. KITAGAWA, W. I. TAYLOR, S. UYEO, and H. YAJIMA, J. chem. Soc. *1955*, 1066.–S. TAKAGI, W. I. TAYLOR, S. UYEO, and H. YAJIMA, J. chem. Soc. *1955*, 4003.

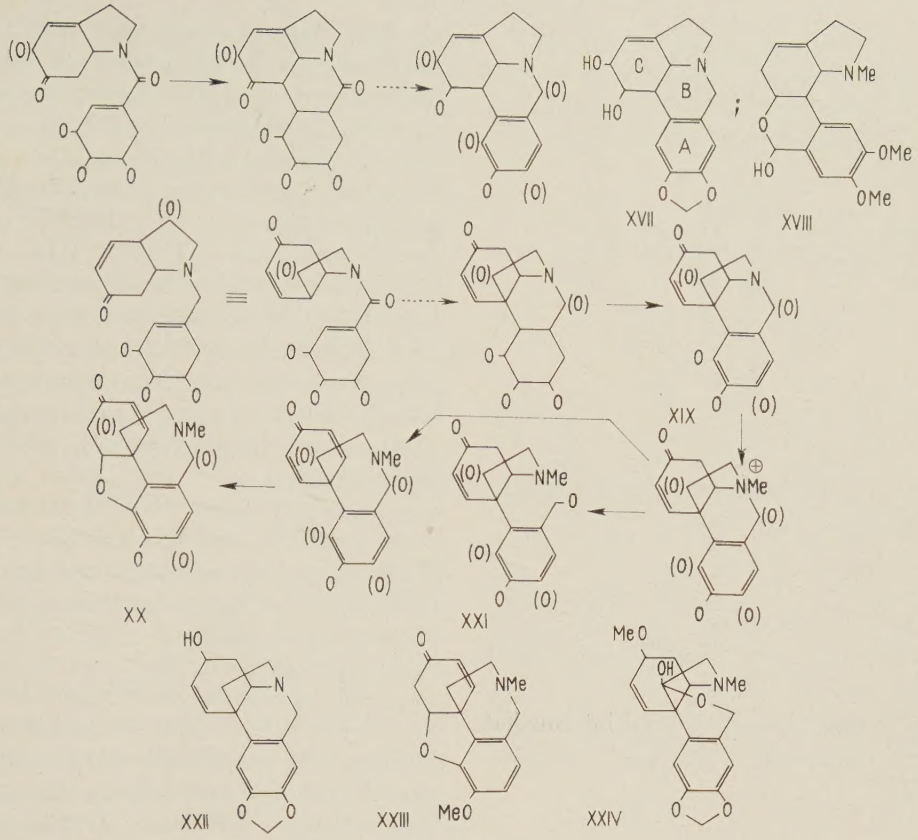
[33] W. C. WILDMAN, J. Amer. chem. Soc. *80*, 2567 (1958).

[34] H. G. BOIT, W. DÖPKE, and A. BEITNER, Chem. Ber. *90*, 2197 (1957).

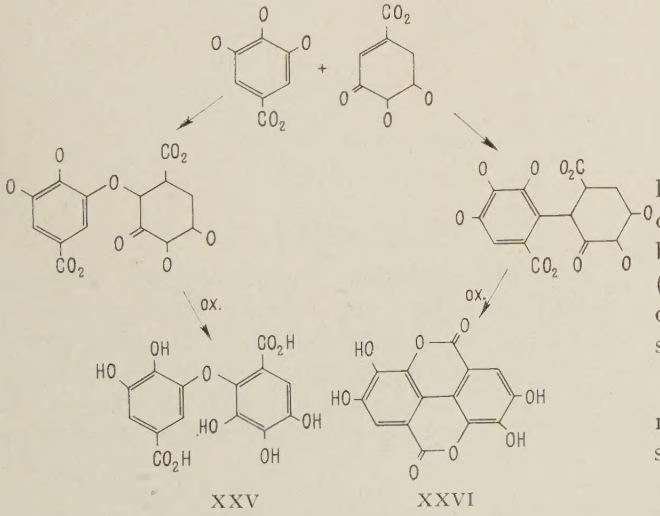
[35] T. IKEDA, W. I. TAYLOR, Y. TSUDA, S. UYEO, and H. YAJIMA, J. chem. Soc. *1956*, 4749.

[36] Since it is not clear whether the benzyl carbon atom on the shikimate unit is the penultimately reduced carboxyl carbon of shikimic acid itself or a formaldehyde unit introduced at an early stage, the following system is an alternative derivative of XV or XVI which requires an internal aldol condensation (rather than a Michael reaction), followed by decarboxylative dehydrations and oxidations, to lead to structures XVII–XXIV:



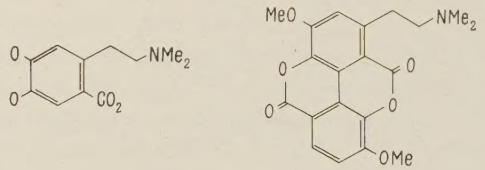


that oxidative phenol coupling of gallate is responsible for the common structure patterns among the tannins[30],[37]. As in the case of the Amaryllidaceae alkaloids above, this need not be the sole biosynthetic pathway. Since undoubtedly gallate is derived from dehydro shikimate by oxidation, the latter also may be involved in the dimerization. In this event the coupling process, a Michael condensation, in the genesis of such compounds as dehydrodigallic acid (XXV) or ellaigic acid (XXVI) would take on a similar appearance to that of the Amaryllidaceae alkaloids:



[37] H. ERDTMAN and C. A. WACHTMEISTER, in *Festschrift Arthur Stoll* (Birkhäuser, Basel 1957), p. 144.

A like interaction of shikimate and the intermediate XXVII, itself obtainable from prephenate (*vide infra*), rather than the previously proposed oxidative degradation of aporphine[38], may represent one of the last stages in the biosynthesis of the alkaloid taspine (XXVIII).



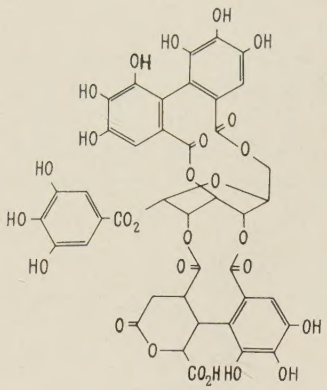
XXVII XXVIII

The hypothesis regarding the Michael addition of hydroaromatic intermediates being preferred in some cases to the oxidative coupling of aromatic systems is best supported by the structure of chebullagic acid (XXIX)[39], wherein the lactone ring appears in a state of oxidation which makes its derivation from dehydro shikimate more likely than from gallate.

Structure XXIX also reveals that a shikimate unit may exist naturally as an acyclic or heterocyclic system. Thus the lactonic acid moiety in XXIX may

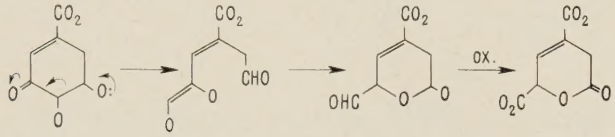
[38] G. F. SMITH, Chem. Soc. Ann. Rep. *53*, 242 (1956).

[39] O. T. SCHMIDT, R. H. HEUSLER, and P. STEPHAN, Ann. Chim. *609*, 186 (1957).

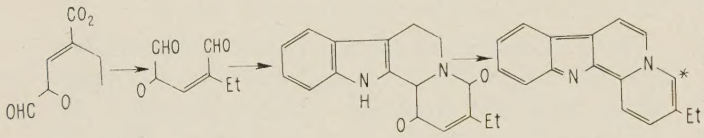


XXIX

arise from a retro-aldol process on dehydroshikimate, followed by oxidation:



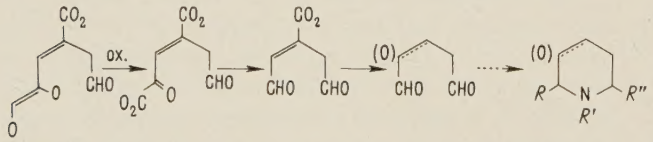
Interaction of a slightly more reduced intermediate with a tryptamine unit leads to the skeleton of flavopereirine (XXX):



XXX

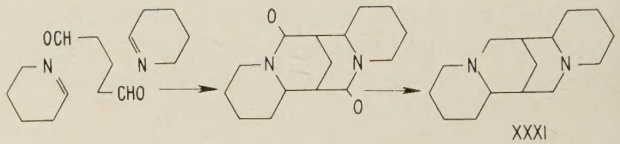
This scheme suggests that a shikimate or shikimate-formaldehyde complex[40] accounts for ring D of the unusual indole alkaloid. A formerly suggested route involving the loss of an acetaldehyde component from a corynantheine derivative[41], presents similar disadvantages to those inherent in an early portrayal of the biosynthesis of the Amaryllidaceae bases[29].

A further consequence of the shikimate cleavage is the possibility of the C_6–C_1 system serving as progenitor of the piperidine unit:

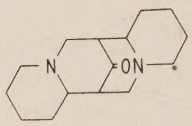


Whereas the construction of the piperidine bases has been associated usually with the metabolism of lysine or close relatives of this amino acid[7,42], nothing unfortunately is known about the origin of the all-important nitrogen atom. In the absence of these data no differentiation can be made between the amino acid and carbohydrate backgrounds of the biointermediates leading to the piperidine nucleus, δ-aminovaleraldehyde, glutaraldehyde, α-oxyglutaraldehyde, glutaconaldehyde and their equivalents. The presence of prephenate units in the alkaloids of black pepper and the Lobelia species suggests that at least in some piperidine bases the carbohydrate scheme may prevail.

In connection with biosynthetic proposals for the various pelletierines and lupanines the interaction of lysine units with formaldehyde and acetone-dicarboxylic acid fragments, or with γ-keto-α,α'-diaminopimelic acid, has been invoked[7,43]. While the unrealistic aspects of the utilization of dicarboxyacetone as a biosynthetic intermediate have been discussed already above, it is noteworthy that its incorporation in the build-up of the lupanines is in violation of known biochemical processes. Irrespective of the exact mode of interaction, the ketonic precursor was considered to be responsible for the formation of the central carbon atoms in the tetracyclic system XXXII. As no lupin alkaloids are oxygenated on the one-carbon bridge, as in XXXII, the latter required reduction to a methylene group. However, such reductions in physiological environment proceed via alcohol and olefin stages, an unallowed route in the present system because of the impossibility of locating a double bond toward the bridgehead of a [3,1,3]-bridge. Instead, the central two rings of an alkaloid such as sparteine (XXXI) must arise from the participation of a third glutaraldehyde unit:



XXXI



XXXII

The C_6–C_2 and C_6–C_3 Units

With the exception of the naturally occurring phenylethylamines, the ephedra bases, a few β-carbolines and substances already discussed above, most alkaloids, whose structures are hypothetically divisible into C_6–C_2 or C_6–C_3 units, are composed of two or more of these fragments. As has been demon-

[40] Just as it is impossible to assign rigorously at this time the location of the shikimate ring rupture or the exact state of oxidation of the biointermediates, it is not certain that the carboxyl group in shikimate becomes the starred atom in flavopereirine. The latter might originate from a formaldehyde unit in analogy with the alternate biosyntheses of colchicine and the Amaryllidaceae alkaloids.

[41] Footnote 14 in E. BÄCHLI, C. VAMVACAS, H. SCHMID, and P. KARRER, Helv. chim. Acta *40*, 1167 (1957).

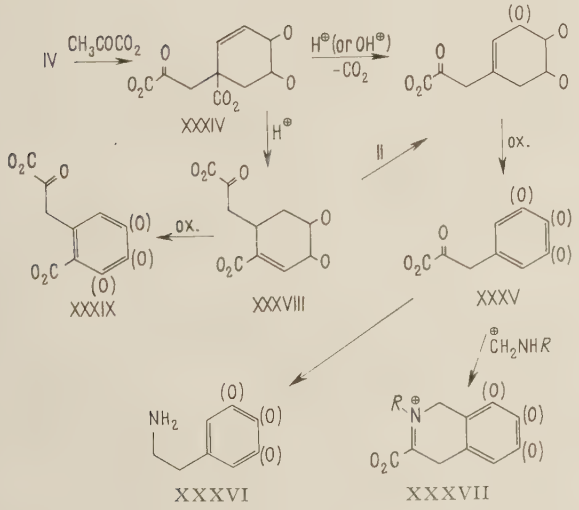
[42] *Cf.* E. LEETE, J. Amer. chem. Soc. *80*, 4393 (1958).

[43] E. ANET, G. K. HUGHES, and E. RITCHIE, Austr. J. Sci. Res. *3A*, 635 (1950).–C. SCHÖPF, G. BENZ, F. BRAUN, H. HINKEL, and R. ROKOL, Angew. Chem. *65*, 161 (1953).–E. E. VAN TAMELEN and J. E. BARAN, J. Amer. chem. Soc. *80*, 4659 (1958).

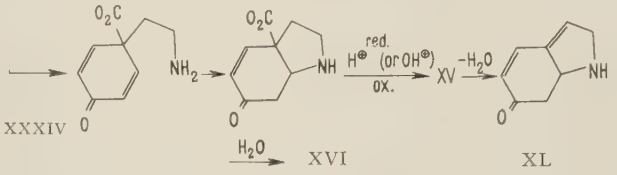
strated in connection with the biosynthesis of indole alkaloids[17], these structural units can originate from hydrated prephenate (XXXIV) through almost every modification of hydration-dehydration, oxidation-reduction, decarboxylation, aldol-retroaldol and Mannich condensations. Chart II portrays the formation of phenylpyruvate (XXXV) and phenylethylamine (XXXVI). A combination of these two substances may lead to products such as aegelin (XXXIII)[44], or alkaloids of the benzylisoquinoline, aporphine, morphine, cularine and bisbenzylisoquinoline types[7]. The creation of the diphenyl and diphenyl ether linkages in the last four classes can occur, as in afore-mentioned cases, at either the aromatic or the hydroaromatic stages, although recent elegant tracer studies have revealed that in the morphine group, at least, the prephenate units are aromatized prior to the coupling process[45]. Formalation of phenylpyruvate (XXXV) yields XXXVII, the precursor of the protoberberine and protopine alkaloids[7]. Since the conversion of XXXIV into a phenylpyruvate (XXXV) system can proceed by direct decarboxylation and aromatization or by prior 1,2-migration of the pyruvate sidechain, the intermediate (XXXVIII) resulting from such rearrangement might be expected to be trapped as an o-carboxyphenylpyruvate (XXXIX). Indeed, the latter serves as the basis for the structures of taspine (XXVII→XXVIII) and the phthalide-isoquinoline alkaloids[46].

XXXIII

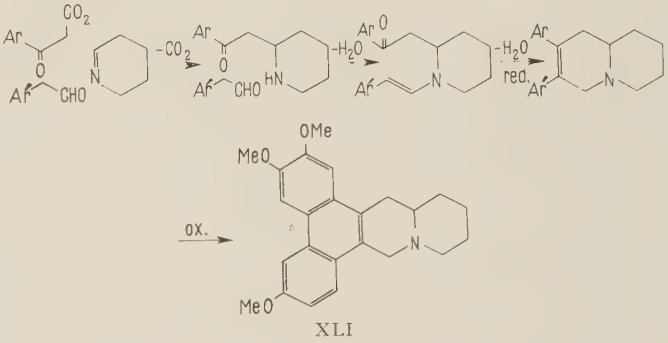
Chart II



Amination of the prephenate unit (XXXV) at an early stage can lead to a ketamine which may act as the progenitor of the hydroindole portion of the erythrina (XL) and Amaryllidaceae alkaloids (XV and XVI)[47]:

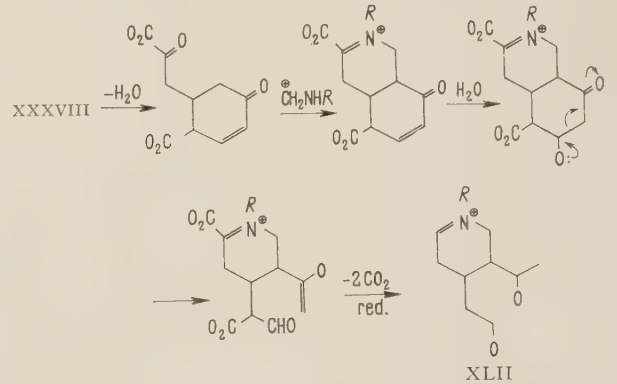


In view of the presence of the active methylene group on the sidechain of the phenylpyruvate unit or its precursors (XXXIV–XXXV) it is not surprising that this position would be the site of further formation of carbon-carbon bonds. Thus direct C-methylation on the sidechain[7] leads to such bases as corydaline, in the protoberberine family, and corycavine, in the protopine class, while aldol condensation at the same site between two prephenate moieties results in the production of alkaloids of the α-naphthaphenanthridine group[48] as well as tylophorine[49] and crytopleurine (XLI)[49]:



XLI

Many of the bases in the vast realm of indole and biosynthetically related alkaloids have been shown to revolve around the all-important intermediate XLII, readily derived from XXXVIII[17]:



XLII

[44] R. N. CHAKRAVARTI and B. DASGUPTA, J. chem. Soc. *1958*, 1580.

[45] A. R. BATTERSBY and B. J. T. HARPER, Chem. & Ind. *1958*, 363.–E. LEETE, Chem. & Ind. *1958*, 977.

[46] This scheme now supersedes the previous suggestion regarding the biosynthesis of this group of alkaloids, i.e. their derivation from the protoberberine bases by oxidation[7]. Sir ROBERT ROBINSON has kindly informed the author of his similar views on this subject.

[47] *Cf.* H. PLIENINGER, Exper. *14*, 57 (1958).

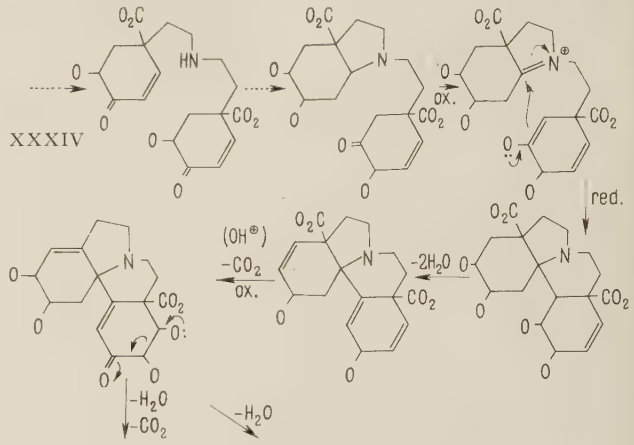
[48] R. H. F. MANSKE in R. H. F. MANSKE and H. L. HOLMES, *The Alkaloids* (Academic Press, Inc., New York 1954), p. 1.

[49] T. R. GOVINDACHARI, M. W. LAKSHMIKANTHAM, K. NAGARAJAN, and B. R. PAI, Chem. & Ind. *1957*, 1484.–P. MARCHINI and B. BELLEAU, Can. J. Chem. *36*, 581 (1958).

Not only are the above intermediates responsible for the genesis of the Yohimbé, Strychnos, Cinchona and Ipecac bases, but they also lay the foundation for the creation of the oxindole[50] and the calebash curare bases[51].

The monotonous consistency with which many alkaloids reveal a twocarbon sidechain in their skeleta strongly hints at the wide distribution of one all-important enzyme, a retroaldolase on the basis of the present theory, which is responsible for the scission of the prephenate unit at a specific site. On this assumption even those alkaloids not based on XLII might be expected to have their prephenate moiety cleaved at the same position. Two such classes appear to exist, the erythrina alkaloids and the indole bases whose dehydrogenation leads to 3,5-dialkylpyridines.

The erythrina bases are made up of two prephenate units, the evolution of one of which has already been illustrated (*cf.* XL). The other structural half is transformed into a phenylethyl group as in the aromatic bases (XLIII) or into a C_5–C_2 lactone as in α-erythroidine (XLIV)[52] and its β isomer. The following indicates their possible biosynthesis:



[50] J. C. SEATON and L. MARION, Can. J. Chem. *35*, 1102 (1957).–J. C. SEATON, R. TONDEUR, and L. MARION, Can J. Chem. *36*, 1301 (1958).

[51] K. BERNAUER, H. SCHMID, and P. KARRER, Helv. chim. Acta *41*, 1408 (1958), and preceding papers.–A. R. BATTERSBY and H. F. HODSON, Proc. chem. Soc. *1958*, 287.

[52] V. BOEKELHEIDE and G. C. MORRISON, J. Amer. chem. Soc. *80*, 3905 (1958).

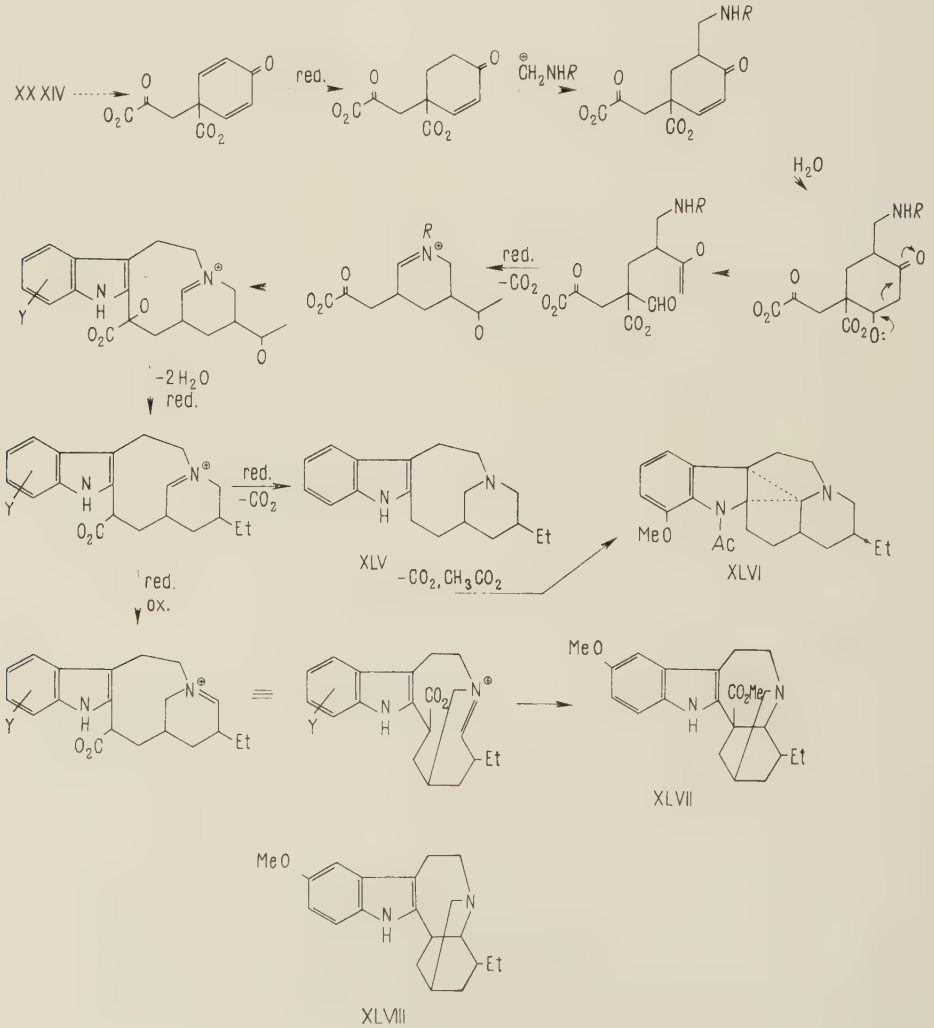
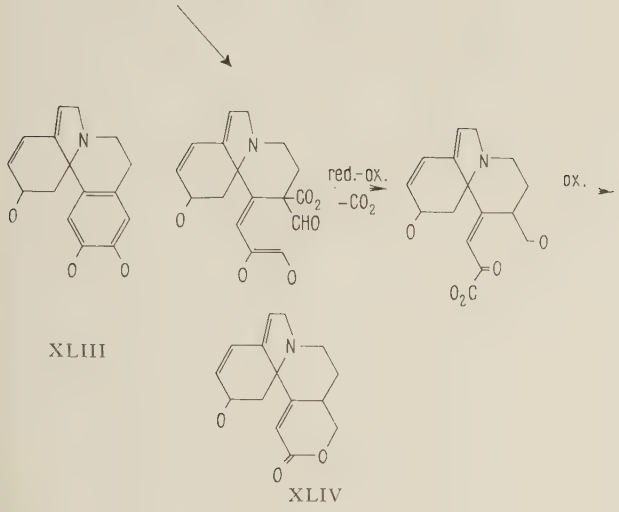


Chart III



XLIII XLIV

The structural features of several of the indole bases isolable from the aspidosperma, iboga and voacanga species reveal their derivation from prephenate, however, not by way of route XXXVIII→XLII. It would seem that in these cases, as in the case of the erythrina bases, the unrearranged skeleton XXXIV itself is the progenitor of the alkaloids under consideration. Formalation and ring opening at now well-determined positions are the initial steps in the pathway to natural products of such structures as quebrachamine (XLV)[53] aspidospermine (XLVI)[53] voacangine (XLVII)[54] and ibogaine (XLVIII)[54]. Their biosynthesis is illustrated in Chart III, pag. 172.

General Observations

The afore-going discussion has attempted to portray the strong likelihood of the biosynthetic derivation of plant alkaloids from carbohydrates, with the exception of the minor intervention of acetate and the possible interposition of ornithine, lysine. This novel unifying theory of alkaloid biosynthesis will now have to be put to a rigorous experimental test, the results of which will be of major interest.

The present theory requires a drastic change in view in connection with at least some hypotheses regarding the reason for the very existence of alkaloids in plants. While it sheds no direct light on this question, it suggests that the answer may be found in the field of the metabolism of plant carbohydrates, rather than amino acids. Thus, for example, should alkaloid production be associated with the plant's mechanism of tangential removal of excess biointermediates from its main metabolic pathways, it would imply that the plant's sugar supply may be excessive. In fact, perhaps this would suggest that alkaloid formation is related to the overabundance of pyruvate, the major intermediate in the shikimate-prephenate scheme.

On the basis of the rapidly emerging patterns of the biosynthesis of plant products, both theoretical and experimental, it is possible to categorize, albeit yet crudely, natural substances into two classes, one based to a large extent on acetate and, hence, on genetically and enzymatically easy routes, and the other founded to a major degree on non-acetate material, i.e. substances farther along in the tricarboxylic acid cycles, and hence, enzymatically difficult, circuitous routes. If it be assumed that the evolution of life processes, i.e. the structure and mechanism of enzymes, through geologic time proceeded from simple to more complex patterns, a correlation of paleobotany with the chemistry of natural products would be on hand. Substances originating from acetate would be expected present in the oldest plants. On this basis the structure of the Lycopodium alkaloid annotinine[55] is no surprise, nor is the discovery of triterpenes from petroleum[56] and coal[57] deposits.

Zusammenfassung

Es wird eine neue Theorie der Alkaloidbiosynthese vorgeschlagen, die alle Alkaloidtypen vereinigt. Die Grundidee dieser Theorie beruht auf der Überlegung, dass die Alkaloide eher mit Kohlehydraten als mit Aminosäuren genetisch verwandt sind. Alle Strukturtypen der Alkaloide sind hypothetisch teilbar in C_4, C_6–C_1, C_6–C_2 und C_6–C_3-Fragmente, und können daher in Beziehung zum Davis-Sprinson-Schema, welches die Biosynthese aromatischer Naturstoffe behandelt, gebracht werden.

[53] Whereas the structures of quebrachamine and aspidospermine have not been fully established, formulas XLV and XLVI are in agreement with available experimental data. These structures were first presented as working models by Professor H. CONROY at the 9th Annual Seminar in the Chemistry of Natural Products, University of New Brunswick, Fredericton, New Brunswick, Canada, October 23–25, 1957 (*cf.* H. CONROY, P. R. BROOK, M. K. ROUT, and N. SILVERMAN, J. Amer. chem. Soc. *80*, 5178 (1958)). Professor SANDOVAL has kindly informed the author of his present use of XLV as a working hypothesis for the structure of quebrachamine (*cf.* B. WITKOP, J. Amer. chem. Soc. *79*, 3193 (1957).–F. WALLS, O. COLLERA, and A. SANDOVAL, Tetrahedron *2*, 173 (1958)).

[54] M. F. BARTLETT, D. F. DICKEL, and W. I. TAYLOR, J. Amer. chem. Soc. *80*, 126 (1958).–W. I. TAYLOR, Exper. *13*, 454 (1957).

[55] K. WIESNER, W. A. AYER, L. R. FOWLER, and Z. VALENTA, Chem. & Ind. 564 (1957).–E. LEETE, Tetrahedron *3*, 313 (1958).

[56] D. H. R. BARTON, W. CARRUTHERS, and K. H. OVERTON, J. chem. Soc. *1956*, 788.

[57] V. JAROLÍN, M. STREIBL, M. HORÁK, and F. ŠORM, Chem. & Ind. *1958*, 1142.

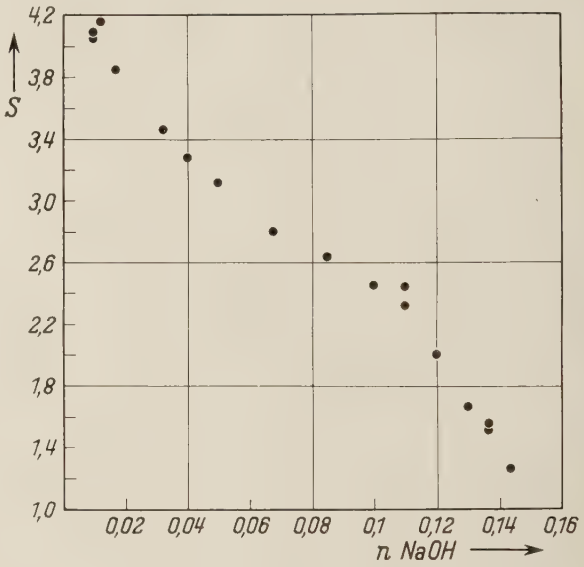
Brèves communications - Kurze Mitteilungen
Brevi comunicazioni - Brief Reports

Les auteurs sont seuls responsables des opinions exprimées dans ces communications. – Für die kurzen Mitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – The editors do not hold themselves responsible for the opinions expressed by their correspondents.

Darstellung und physikochemische Charakterisierung der Peptidketten des Tabakmosaikvirus

Endgruppen- und Röntgenstrukturanalysen haben ergeben, dass der Proteinmantel des Tabakmosaikvirus ($M \approx 40 \cdot 10^6$) aus ca. 2300 Peptidketten besteht. Es ist bisher jedoch noch nicht gelungen, das Virus bis zu den einzelnen Peptidketten zu spalten und deren Grösse mit physikochemischen Methoden zu bestimmen. Es gibt meines Wissens nur zwei Literaturangaben[1,2] über den Versuch einer Virusspaltung, welche die Grösse des sogenannten A-Proteins ($M \approx 90–100 \cdot 10^3$) unterschreitet. Dabei sind die Zentrifugierungen in sehr ionenarmen Lösungen durchgeführt und daher mit dem durch den primären Ladungseffekt bedingten Fehler behaftet; Diffusionsmessungen fehlen gänzlich. Bisher nicht veröffentlichten Versuchen[3] zufolge kann man nach Phenolspaltung des Virus und Lösen in verdünnter Natronlauge unter Ausschluss der Luftkohlensäure ein Protein mit $M \approx 18\,800$ erhalten. – Für den Vergleich zwischen elektrophoretischen und biochemischen Untersuchungen an unseren TMV-Stämmen[4–5] erwies es sich als notwendig, das Virusprotein bis zu den Peptidketten abzubauen. Es werden im folgenden Methoden für die Spaltung des TMV beschrieben. Über einen Teil der Experimente wurde bereits auf dem IV. Internationalen Biochemie-Kongress in Wien berichtet[6]. – Die Grösse der Spaltprodukte wurde durch Sedimentations- und Diffusionsmessungen bestimmt; die Zentrifugierungen erfolgten mit der Meyerhoffschen Unterschichtungszelle in einer luftgetriebenen analytischen Phywe-Ultrazentrifuge bei 51 000 U/min und 20°C. Alle angegebenen Werte sind hinsichtlich Viskosität, Dichte, Temperatur und ggf. Druck auf Wasser und 20° korrigiert, wobei die Korrekturwerte entweder experimentell bestimmt oder Tabellen entnommen sind. – Der Virusabbau erfolgte im einzelnen nach folgenden Methoden:

1. In m/50 Phosphatpuffer pH 7 befindliches TMV wurde bei 4°C 12–15 min lang mit wassergesättigtem Phenol[7] geschüttelt, nach Zentrifugation die Phenolphase mit etwas mit Natriumacetat gesättigtem Methanol versetzt, das Protein mit Methanol gefällt und je 2mal mit Methanol bzw. Äther gewaschen. Das getrocknete Proteinpulver (0,2%) wurde in 0,12% *Natriumdodekylsulfat* (NDS) enthaltendem Veronalpuffer (pH 8, m/50) gelöst (3 min; 70°C). Unter Berücksichtigung der Veränderung des partiellen spezifischen Volumens durch die Bindung von NDS an das Protein ergibt sich: $s_{20,w} = 1{,}9$ S und $D_{20,w} = 8{,}6 \cdot 10^{-7}$ cm²/sec. Wird NaCl ($\Gamma = 0{,}1$) zu der obigen Lösung nach dem Erkalten hinzugefügt und dialysiert, so betragen $s_{20,w} = 2{,}1$ S und $D_{20,w} = 7{,}5 \cdot 10^{-7}$ cm²/sec. Ähnliche Werte erhält man, wenn TMV bei pH 8 in m/50 Veronalpuffer, dem 0,05% NDS zugesetzt wurde, für 5 min auf 80–85°C erhitzt und nach dem Abkühlen der Lösung NaCl zugegeben wird.



$s_{20,w}$-Werte nach 10–15stündiger Dialyse des unter Nr. 2 beschriebenen TMV-Proteins (0,5%) gegen verschiedene NaOH-Konzentrationen; Ionenstärke durch NaCl-Zusatz konstant erhalten.

2. TMV wurde mit Phenol gespalten und das Proteinpulver in n/10 NaOH durch kurzes Erwärmen auf 40° gelöst. Nach 10–15stündiger Dialyse bei 20° in einem geschlossenen Erlenmeyer gegen 0,01 bis 0,15 n Natronlauge, der zur Vermeidung des primären Ladungseffektes NaCl bis zur Gesamtionenstärke $\Gamma = 0{,}15$ zugefügt wurde, trat bei der Zentrifugierung immer nur ein einheitlicher Gradient auf; in der Abbildung ist $s_{20,w}$ in Abhängigkeit von der NaOH-Konzentration aufgetragen. Zusatz von NaCl über $\Gamma = 0{,}1$ hinaus verändert den $s_{20,w}$-Wert nicht, während bei niedrigen Ionenstärken ein deutlicher Einfluss des zugesetzten NaCl auf $s_{20,w}$ vorhanden ist, wie in mehreren Versuchsreihen bei konstanter NaOH-Konzentration und variierendem Na-Cl-Zusatz bei gleich langer Dialyse gezeigt werden konnte. Aus Viskositäts- und Diffusionsmessungen geht hervor, dass die aus der Abbildung ersichtliche Abhängigkeit des S-Wertes von der angewendeten NaOH-Konzentration nicht auf eine Entfaltung, sondern eher auf eine partielle Hydrolyse des Proteins zurückzuführen ist, dabei können sekundäre Bindungen zwischen den Peptidketten vorhanden sein. Diese Folgerung wird dadurch gestützt, dass mit zunehmender Dialyse-Zeit (über mehrere Tage verfolgt) $s_{20,w}$ einer bestimmten Proteinlösung abnimmt, und zwar um so schneller, je höher die NaOH-Konzentration ist. Als weiterer Beweis für die Spaltung lässt sich anführen: Dialyse gegen n/10 NaOH für 10 h ergibt 2,4 S, nach weiterer 10stündiger Dialyse dieser Lösung gegen n/50 NaOH erhöht sich der Wert nicht auf 3,5 S, den eine 20 h gegen n/50 NaOH dialysierte Lösung ergibt, sondern er beträgt nur noch 2,3 S. Die Verringerung des S-Wertes

[1] R. T. Hersh und H. K. Schachman, zitiert bei: H. Fraenkel-Conrat und B. Singer, J. Amer. chem. Soc. *76*, 180 (1954).
[2] P. Newmark und R. W. Myers, Fed. Proc. *16*, 226 (1957).
[3] A. Anderer, persönl. Mitteilung, Dez. 1958.
[4] E. Kramer und H. G. Wittmann, Z. Naturforsch. *13b*, 30 (1958).
[5] H. G. Aach, Z. Naturforsch. *13b*, 425 (1958).
[6] H. G. Wittmann und E. Kramer, Proc. IV. Intern. Bioch. Kongr. Wien (1958), im Druck.
[7] H. Schuster, G. Schramm und W. Zillig, Z. Naturf. *11b*, 339 (1956).

ist demnach ein irreversibler Prozess. Folgendes Ergebnis weist in dieselbe Richtung: Erwärmung des in n/10 NaOH gelösten Proteins für 10 min auf Temperaturen zwischen 40–100° und anschliessende Dialyse (10 h; n/10 NaOH; 20°) ergibt einen deutlichen Abfall von 2,4 auf 0,9 S. – Wird die Proteinsubstanz direkt in NaOH der gewünschten Stärke gelöst und nach NaCl-Zugabe ($\Gamma = 0{,}15$) sofort zentrifugiert, so erhält man in n/50 bis n/7 NaOH Werte um 2,8 S; das heisst, dieser Sedimentationskoeffizient ist höher, als für Peptidketten mit M $\approx$ 16000–18000 erwartet werden muss. – Man kann aus den genannten Messungen in verdünnter NaOH nicht eindeutig auf ein bestimmtes M-Gewicht schliessen, da unter diesen Bedingungen mit dem Ablauf sekundärer Prozesse zu rechnen ist.

3. 60 Volumenteile bis kurz über dem Erstarrungspunkt gekühlter 99prozentiger *Essigsäure* wurden mit 30 Teilen Viruslösung (in H_2O) bei 4° gemischt[8] und 15 min unter leichtem Schütteln stehen gelassen, die ausgefallene RNS abzentrifugiert und zu dem Überstand 10 Volumenteile 2,5 m NaCl zugesetzt. Auf c = 0 extrapoliert, betragen s_{20}, w = 1,25 S und D_{20}, w = $7{,}9 \cdot 10^{-7}$ cm²/sec, woraus sich M = 17800 errechnet.

4. TMV wurde 24 h bei 4° gegen m/10 Glykokollpuffer pH 10,5 dialysiert, das Protein zweimal mit $(NH_4)_2SO_4$ gefällt und gegen m/50 Veronalpuffer pH 8,0 dialysiert. UV-Absorptionsmessungen in Phosphatpuffer pH 7 gaben über die Reinheit des Proteinpräparates Aufschluss. Um die bei der Denaturierung durch die folgende *Pyridin*-Behandlung freiwerdende SH-Gruppe des Proteins zu blockieren und dadurch eventuelle sekundäre SH-Reaktionen zu verhindern, wurde p-Chloromercuribenzoat (pCMB) in einer Endkonzentration von m/1000 zugegeben. 70 Volumenteile der Proteinlösung (in Veronalpuffer + pCMB) wurden mit 30 Volumenteilen Pyridin gemischt und vor der Zentrifugierung mit 10 Teilen m/1 NaCl versetzt. Als Ergebnis wurde s_{20}, w = 1,35 S und D_{20}, w = $7{,}2 \cdot 10^{-7}$ cm²/sec, beide auf c = 0 extrapoliert, sowie M = 17300 erhalten. – Zu ähnlichen Resultaten kommt man, wenn Virus tropfenweise zu der pCMB enthaltenden Pyridin-Wasserlösung unter Schütteln hinzugegeben und anschliessend NaCl ($\Gamma = 0{,}1$) zugesetzt wird. Die Virusstäbchen zerfallen in der Pyridinlösung unter Denaturierung sofort in die Untereinheiten.

Fräulein Ingrid Hindennach und Karin Sandelin danke ich für die wertvolle Mitarbeit bei der Durchführung der Versuche.

H. G. Wittmann

Max-Planck-Institut für Biologie, Tübingen, 22. Dezember 1958.

Summary

Highly purified tobacco mosaic virus was split by different methods, the ribonucleic acid removed and the size of the protein sub-unit determined by sedimentation and diffusion. The value for the molecular weight of a polypeptide chain of the tobacco mosaic virus was calculated to be 17–18000.

[8] H. Fraenkel-Conrat, Virol. *4*, 1 (1957).

Über die Entstehung von Orientierungen in Nylon 6 durch Kontakt mit der Spaltfläche von KCl

An dem Beispiel der orientierten Aufwachsung von Polyäthylen auf NaCl haben J. Willems und I. Willems[1] erstmalig gezeigt, dass auch hochmolekulare organische Stoffe orientiert zu verwachsen vermögen. Diese Verwachsung wurde auf lichtmikroskopischem Wege gefunden. Das Verwachsungsgesetz und die kristallographische Natur der orientiert aufgewachsenen Teilchen des Polyäthylens wurden von Fischer[2] mit Hilfe des Elektronenmikroskops aufgeklärt.

Bei weiteren systematischen Aufwachsungsversuchen mit organischen hochmolekularen Stoffen der verschiedensten Art unter Verwendung mannigfaltiger Trägerkristalle wurden keine mit dem Lichtmikroskop erkennbaren orientierten Teilchen der hochmolekularen Stoffe gefunden. Es wurde deshalb eine Methode ausgearbeitet, die Orientierungserscheinungen an hochmolekularen Stoffen an Grenzflächen auf lichtmikroskopischem Wege auch dann erkennen lässt, wenn die orientierten Teilchen an sich unter der Sichtbarkeitsgrenze des Lichtmikroskops liegen. Diese Methode besteht darin, dass der hochmolekulare Stoff als dünner Film auf die Trägerfläche in Lösung oder in der Schmelze aufgebracht und nach dem Ablösen des Trägers durch Aufwachsungsversuche mit geeigneten Gastsubstanzen auf der Filmfläche des hochmolekularen Stoffes festgestellt wird, ob eine Orientierung und gegebenenfalls welcher Art eingetreten ist. Nähere Einzelheiten über dieses «Abdruckverfahren», bei dem die Trägerfläche sozusagen als Matrize wirkt, werden an anderer Stelle veröffentlicht werden.

Nach diesem Abdruckverfahren gelang es, durch Aufbringen einer Lösung von Nylon 6 (Polyamid der ε-Aminocapronsäure) in Kresol auf (100) von KCl bei 195°C, einen Film zu erhalten, auf dessen Oberfläche nach dem Ablösen des KCl-Kristalls mit Wasser die nadelförmigen Kriställchen von aufgedampftem Pentachlorphenol sich in 4 Stellungen ausgezeichnet orientieren. Diese 4 Stellungen sind um 45° gegeneinander gedreht.

Elektronenmikroskopisch wird die weitere Aufklärung dieser Orientierung fortgeführt.

J. Willems

Krefeld, Tiergartenstrasse 21, 1. Oktober 1958.

Summary

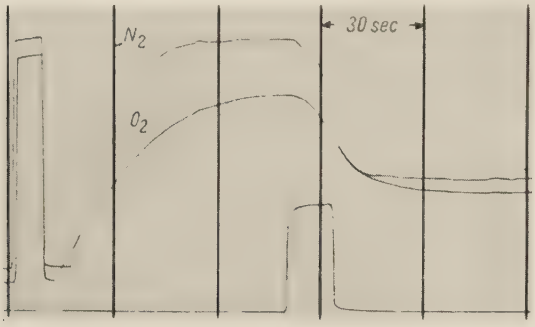
Oriented overgrowth of pentachlorophenol was obtained on a thin film of Nylon 6 produced in contact with (100) of KCl.

[1] J. Willems und I. Willems, Exper. *13*, 465 (1957).
[2] E. W. Fischer, Faraday Soc. Discuss. 1958 (im Druck).

A System in the Mammalian Skin Sensitive to X Irradiation and Insensitive to the Oxygen Effect

Molecular oxygen increases the effects of X or γ-irradiation. It has been questioned whether or not the substances which protect living organisms against X-irradiation act by reducing the oxygen pressure in the tissues,

Brinkman and Lamberts[1] and Lamberts[2] have described a phenomenon abolished by the radioprotector cystamine discovered by Bacq and Herve[3]. We have observed that it is not sensitive to the presence of O_2.



Pressure records. One injection needle is supplied with normally oxygenated saline, the other with oxygen-depleted solution. In each case, calibration of the apparatus to 100 mm Hg; then the pressure is slowly built up until a plateau is reached. The lower curve indicates the time of X-irradiation (175 r/sec). Both pressures fall in the same manner.

Thanks to the help of Dr. Lamberts, we have built in Liège the apparatus used by the Dutch scientists. It consists of two very fine injection needles (no. 20) inserted in the skin of a young adult mammal (rat, man) in the deep layer of the dermis where the mucopolysaccharide fibres are numerous. One builds a pressure of about 100 mm Hg by slow constant injection of 0·9% NaCl solution; the pressure is limited by a system of valves and registered on photographic paper by means of electronic tubes which transform pressure in potential changes. The skin is irradiated locally just above the tip of the needle with a soft X-ray beam (30 kV, 15 mA, 175 r/sec); after a latency of only 1 sec, the pressure falls, presumably because hyaluronic acid depolimerizes. This effect is observed in a living or a recently dead rat. We have confirmed Brinkman and Lambert's observations. In order to be sure that oxygen is not involved, we have worked with rats anaesthetized by Nembutal, killed in a plastic box where a slight pressure of pure nitrogen was maintained. The oxygen dissolved in the injected solution was carefully removed by a vacuum pump and 3 or 4 washings with pure nitrogen. The effect of X-irradiation in this condition of strict lack of oxygen is the same as that seen when no precautions are taken to exclude oxygen (Fig.). We have seen that the radioprotector tryptamine (HCl, 0·01 M) inhibits the fall of pressure just as cystamine does.

Thus the oxygen effect in this case cannot explain the action of cystamine or tryptamine which is better interpreted by the theory of trapping of free radicals as proposed by Alexander, Bacq *et al.*[4].

Z. M. Bacq, P. Ciccarone*, and J. Renson

Laboratories of General Pathology and of Civil Security, University of Liège (Belgium), November 28, 1958.

[1] R. Brinkman and H. B. Lamberts, Nature *181*, 774 (1958).
[2] H. B. Lamberts, *Chemische bescherming tegen beschadigende bestraling* (Thesis, Groningen 1958), p. 124.
[3] Z. M. Bacq and A. Herve, Bull. Acad. Roy. Méd. Belg. [VIe série] *17*, 13 (1952).
[4] P. Alexander, Z. M. Bacq *et al.*, Radiation Res. *2*, 392 (1955).
* Fellow of the Belgo-Italian Commission for Cultural Relations.

Résumé

La chute de pression sous l'influence des rayons X dans une poche de solution saline injectée dans la peau du rat (phénomène décrit par Brinkman et Lamberts) se produit aussi en l'absence d'oxygène. L'action de certains radioprotecteurs, mise en évidence par cette technique, est donc également indépendante de l'oxygène.

Occurrence of a Pigment Layer in *Gastrothylax crumenifer* (Creplin, 1847)

Pigments in helminths have been known for a long time but during the last decade or two some of these have been shown to resemble haemoglobin (Wharton[1], Stephenson[2], van Grembergen[3], Davenport[4], and Rogers[5]). The present paper gives an account of a pigmented layer in an amphistome, *Gastrothylax crumenifer* (Creplin, 1847) commonly parasitizing the rumen of cattle.

Fig. 1. – Photograph of a thick transverse section of *Gastrothylax crumenifer* to show the pigment layer lining the cavity of the ventral pouch.

The body of this amphistome appears red or pink in fresh condition when removed from the rumen, but in a transverse section of this amphistome (Fig. 1) the colour can be traced to its ventral pouch which is a large cavity showing a red coloured layer. This pigmented layer lies just over the layer of the oblique muscles next to the general parenchyma and appears to be slightly vacuolated. As the pigmented layer extends along the length of the ventral pouch, the entire body of the amphistome appears red. The pigmented layer can be peeled off from the cut surface in an amphistome longitudinally bisected through its ventral pouch (Fig. 2) and parts of the body of the amphistome without this layer appear almost greyish-white. The pigment seems to be concentrated in this layer, there being little dispersal of the pigment elsewhere.

Extraction of this pigment from amphistomes was done by a simple method. Live worms along with some contents of the rumen maintained at about 37°C in a thermos flask were brought to the laboratory from the slaughter house. The worms were thereafter thoroughly rinsed in distilled water and then cut longitudinally into two halves across the cavity of the ventral pouch. The cut worms were

[1] G. W. Wharton, J. Parasit. *27*, 81 (1941).
[2] W. Stephenson, Parasitology *38*, 128 (1947).
[3] G. van Grembergen, Enzymologia *13*, 241 (1949).
[4] H. E. Davenport, Proc. R. Soc. London *B 136*, 255 (1949). – R. F. H. Freeman and J. Llewellyn, J. Mar. Biol. Ass. *37*, 435 (1958).
[5] W. P. Rogers, Australian J. sci. Res. *B 2*, 287 (1950). – D. Keilin, Proc. R. Soc. London *B 98*, 312 (1925).

washed quickly with two to three changes of distilled water and then left in distilled water in a frigidaire. Within 48–72 h most of the pigment was extracted in water and the amphistomes became colourless. Peelings of the pigmented layer were obtained from the ventral pouch of the amphistome and treated in a similar way. This gave the pigment solution with little contamination with the animal tissue fluid. In fact this latter method was more

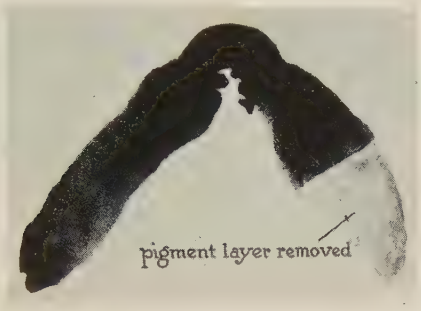


Fig. 2. – Photograph of a specimen of *Gastrothylax crumenifer* split longitudinally into two halves to show the pigment layer lining the ventral pouch. Part of the layer removed in one half to show the grey body.

satisfactory as most of the pigment is confined to the lining of the ventral pouch. The pigment solution was centrifuged and the supernatant clear red fluid was separated and stored in a frigidaire. When 4% trichloracetic acid or 90% alcohol was added to this fluid, a brownish red precipitate, mainly of protein, was formed and a colourless solution was left. This suggested the association of pigment with protein. In another experiment, glacial acetic acid was added to the aqueous solution of the pigment and the mixture shaken in a test tube with a little ether and allowed to stand for some time. Three distinct layers or

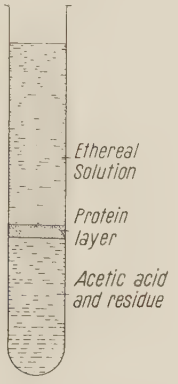


Fig. 3. – Diagram to show the three layers after treatment of the pigment solution with acetic acid and ether.

columns of liquid (Fig. 3) appeared in the test tube (Hawk, Oser, and Summerson[6]). The upper column contained a reddish etherial solution of the pigment, below this was a narrow zone of precipitated protein which was devoid of colour (the colour having been isolated from the protein by the action of glacial acetic acid and ether), and the bottom column was a mixture of acetic acid and residual water. Both the aqueous and etherial solutions were tested for the presence of iron. A few drops of these solutions were evaporated to dryness and the residue ignited and extracted with dilute hydrochloric acid. A distinct blue coloration was developed in the solution on addition of a drop of dilute potassium ferrocyanide solution, thereby indicating the presence of iron. No such coloration was obtained in the controls.

When a dried film of the aqueous solution of the pigment on a glass slide was flooded with a drop of glacial acetic acid containing a few crystals of potassium iodide and examined under a polarizing microscope, minute boat-shaped birefringent crystals of protoiodoheme were observed which resembled protoiodoheme crystals prepared from human blood (Glick[7], p. 62). In another experiment, a small drop of the aqueous solution of the pigment greatly diluted with distilled water was put on a glass slide. Then a fraction of a drop of 0·75% NaCl was added to it and the fluid carefully evaporated to dryness over a microburner. A cover glass was put over the dried spot and a drop of glacial acetic acid was run underneath. The slide was warmed gently until the formation of gas bubbles. Another small drop of glacial acetic acid was run beneath the coverslip and the preparation on cooling was examined under the microscope. Parallelogram-shaped hemin crystals were observed which also resembled the hemin crystals prepared from human blood (Hawk, Oser, and Summerson[6]).

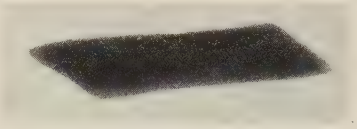


Fig. 4. – Photomicrograph of a greatly enlarged parallelogram-shaped hemin crystal obtained from the pigment of *Gastrothylax crumenifer*.

Pigment extracted from 20 amphistomes in about 2 ml of water and diluted 1:4 was subjected to a spectroscopic examination by Hilger's spectrograph and a microspectroscope. This revealed two absorption bands, a-band at 5735–5850° Å and b-band at 5410–5535° Å (diffuse). In order to determine the peaks within these ranges, a Bausch and Lomb spectrophotometer was employed which indicated the absorption peaks at 5750 and 5450° Å respectively, corresponding to the two bands. Addition of Takayama's fluid (Hawk, Oser, and Summerson[6]) to the aqueous solution of the pigment did not materially alter the position of the original two bands; but, when the mixture was heated and examined by a microspectroscope, two characteristic bands different from the normal, one at 5550–5650° Å (sharp) and the other 5200–5300° Å (faint) were seen. These apparently conform to the characteristic bands of pyridine haemochromogen produced when the haem pigments are treated with Takayama's fluid.

The pigment obtained from the lining of the ventral pouch of *Gastrothylax crumenifer* is associated with protein. This fact is proved firstly by the coloured protein precipitate obtained after treatment with trichloracetic acid, secondly by the fact that the pigment appears to dissolve more readily in ether after acetic acid treatment, and thirdly by the liberation of the coloured product from the protein during this treatment. That the pigment might probably be of a haemin type was also made clear when an aqueous solution of the pigment was subjected to a spec-

[6] P. B. Hawk, B. L. Oser, and W. H. Summerson, *Practical Physiological Chemistry* (Toronto 1954).

[7] D. Glick, *Techniques of Histo- and Cytochemistry* (New York 1949).

troscopic examination. This revealed the usual two absorption maxima at 5750 and 5450. Å. With the addition

Fig. 5.—A graph showing the absorption peaks of the pigment solution of *Gastrothylax crumenifer*.

of Takayama's fluid and application of heat, the characteristic pyridine haemochromogen bands at 5200–5300° Å (faint) and 5550–5650° Å (sharp) were also detected on spectroscopic examination. The chemical detection of traces of iron and the positive deposit of boatshaped protoiodoheme and parallelogram-shaped hemin crystals comparable to those obtained from human blood, suggest a haem base in this pigment. The solubility tests also point to a non-carotenoid nature of the pigment. It is, however, not possible for the writer at this stage to explain the peculiar disposition of the pigmented layer in the ventral pouch and its possible role in the physiology of this amphistome.

M. B. Lal

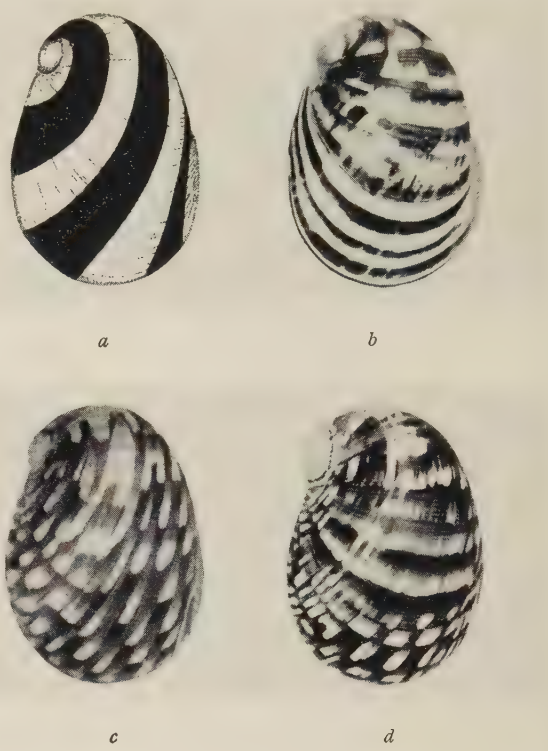
Department of Zoology, The University, Lucknow (India), December 1, 1958.

Résumé

Il s'agit de l'étude d'une couche rouge pigmentée formant la surface intérieure de la poche ventrale de l'amphistome *Gastrothylax crumenifer* (Creplin, 1847). Les expériences de solubilité démontrent la nature non-caroténoide de ce pigment. L'examen spectroscopique et les expériences chimiques prouvent la présence d'une base (haem) dans le pigment.

Farbmusterumschlag auf der Molluskenschale

Zahlreiche Untersuchungen an Farbmustern von Schmetterlingen, Vögeln und Säugern haben gezeigt, dass die tierischen Farbmuster geeignete Modelle für eine entwicklungsphysiologische Analyse einfacher Gliederungsvorgänge sind (Henke[1]). Die Farbmuster der Molluskenschale sind dagegen aus Mangel an einem geeigneten Laboratoriumstier bisher wenig berücksichtigt worden. Ihre morphologische und experimentelle Untersuchung konnte neuerdings aufgenommen werden, nachdem die Züchtung der einheimischen Flussdeckelschnecke *Theodoxus fluviatilis* L. (abgekürzt *Th. fl.*) gelungen war.



Farbmuster von *Theodoxus fluviatilis* aus der Werra. *a–c:* die drei Farbmustertypen (Erläuterungen siehe Text); *d:* sukzessiver Musterumschlag zwischen dem synchronen Querstreifenmuster und dem phasenlängenkonstanten Längsmuster. Vergrösserung 1:5.

Das Farbmuster von *Theodoxus* gehört der zwischen Periostracum und Hauptkalkschicht gelegenen Musterkalkschicht an (Becker[2]). In ihr finden sich im Bereich dunkler Schalenteile braune Einlagerungen, das Schalenpigment[2], und im Bereich heller Schalenteile farblose Einlagerungen, die Schalengranula. Die Musterkalkschicht erfährt einen Zuwachs nur längs des Schalenrandes und ist einer gesonderten Bildungszone des Mantelrandepithels zuzuordnen[2]. Aus der gleichbleibenden oder wechselnden Verteilung von pigment- und granulabildenden Zellbereichen dieses Musterkalkschicht-Epithels fügt sich im Wachstumsverlauf das Schalenmuster von *Theodoxus* zusammen. Bei *Th. fl.* können bisher drei Farbmustertypen gegeneinander abgegrenzt werden, denen qualitativ voneinander verschiedene Gliederungsvorgänge zugrunde liegen. 1. Das Sektorenmuster (Abb. 1*a*, halbschematisch) ist mit dem Muster der einheimischen Bänderschnecken vergleichbar. Es entsteht, wenn die Verteilung der bevorzugt pigment- und granulabildenden Bereiche bereits frühzeitig in der Entwicklung determiniert und anschliessend während des weiteren Schalenwachstums unverändert beibehalten wird. 2. Das synchrone Querstreifenmuster (Abb. 1*b*) erscheint auf der Schalenfläche, wenn Pigment- und Granulabildung zeitlich nacheinander längs des gesamten Schalenrandes mit-

[1] K. Henke, Naturwissenschaften *35*, 176, 203, 239 (1948).

[2] K. Becker, Biol. Zbl. *68*, 263 (1949).

einander abwechseln. 3. Das phasenlängenkonstante Längsmuster (abgekürzt auch Tropfenmuster) zeigt eine Ordnung von hellen Flecken oder Tropfen auf dunklem Feld (Abb. 1*c*). Es bildet sich vermutlich bei der Überlagerung zweier Gliederungsvorgänge, und zwar eines Simultanrhythmus (Henke[1]), welcher die gleichabständige Längsgliederung der hellen und dunklen Schalenteile bedingt, und eines Ausbreitungsvorganges, der von den Pigmentbereichen ausgeht und die alternierende Ordnung der hellen Schalenteile herstellt.

Bei der Untersuchung der Farbmuster-Variabilität von *Th. fl.* liessen sich in der Werrapopulation Schalen auffinden, die einen Umschlag des Farbmusters zwischen zwei Mustertypen, zwischen dem Querstreifenmuster und dem Tropfenmuster, zeigten (Abb. 1*d*). Ein möglicherweise gleichzeitig vorhandenes Sektorenmuster wird bei diesem sukzessiven Musterumschlag unverändert beibehalten. Reihenfolge und Zeitpunkt, in denen sich der Umschlag ereignet, sind bei der Durchsicht eines grösseren Freilandmaterials scheinbar willkürlich. Hin und wieder kann der Musterumschlag auch mehrmals erfolgen; er ist reversibel. Unter konstanten und wechselnden Aufzuchtbedingungen im Laboratorium liess sich an einem umfangreichen Material beweisen, dass bei der weit überwiegenden Anzahl der untersuchten Schnecken der Musterumschlag auf Grund spezifischer Veränderungen in der Salzzusammensetzung des Aussenmediums auslösbar ist (weitere berücksichtigte Aussenfaktoren: Temperatur und Ernährung). Im einzelnen konnte mittels synthetischer Zuchtlösungen gezeigt werden, dass Unterschiede in der relativen Ionenzusammensetzung – vorwiegend im Ca/Mg-Verhältnis – wesentlich sind, während Differenzen in der Gesamtkonzentration (bei gleicher Ionenzusammensetzung) offenbar keine Rolle spielen. So dominierte bei einem Ca/Mg-Verhältnis ≥ 1 (bezogen auf die Äquivalentprozente) das Tropfenmuster und bei einem Verhältnis $\leq 0{,}5$ das Querstreifenmuster. Damit liess sich auch der im Freiland beobachtete Musterumschlag der weit bis in das Brackwasser vordringenden Schnecke deuten, da von der Werra entsprechende Schwankungen der Salzzusammensetzung bekannt sind. So treten infolge einer Abwassereinleitung der Kaliindustrie im Bereich der unteren Werra Salzgehalte bis maximal $14^0/_{00}$ auf (Schmitz[3]). Gleichzeitig mit diesen Salzgehaltsschwankungen ändert sich entsprechend der verschiedenen Relativzusammensetzung von Süss- und Salzwasser auch die relative Ionenzusammensetzung.

Der sukzessive Musterumschlag lässt einmal erkennen, dass die dem Querstreifen- und dem Tropfenmuster zugrunde liegenden Gliederungsfaktoren innerhalb der gleichen Reaktionsnorm liegen können. Zum anderen zeigt er, dass das Schalenmuster dieser beiden Mustertypen im Gegensatz zum Sektorenmuster für alle Schalenabschnitte nacheinander und unabhängig determiniert wird. Je nach den im Aussenmedium herrschenden spezifischen Ionenverhältnissen, welche bei *Th. fl.* auf das Innenmedium einwirken müssen, greifen nur die Gliederungsfaktoren eines der beiden Farbmustertypen in die Differenzierung ein. In möglichen Zwischenstufen treten (hier nicht erwähnte) Überlagerungen auf, bei denen die Gliederungsfaktoren beider Mustertypen gleichzeitig die im Mantelrand ablaufenden Gliederungsvorgänge lenken.

Eine ausführliche Mitteilung der Untersuchungen über die Farbmuster von *Th. fl.* wird vorbereitet.

D. Neumann

Zoologisches Institut der Universität Göttingen, 15. Januar 1959.

[3] W. Schmitz, Vom Wasser *23*, 113 (1956).

Summary

The variability of the shell pattern of *Theodoxus* (Werra-population) is reduced to three significant types, which are based on different processes of pigment formation of the mantle-edge. A change between the 'synchronen Querstreifen'-pattern and the 'phasenlängenkonstanten Längsstreifen'-pattern is described. This change can be achieved at any time on the growing shell by altering the ionic composition of the external medium, i.e. these two types of pattern are determined stage by stage in the successive parts of the shell.

Guatambuine, an Alkaloid of *Aspidosperma longepetiolatum* Kuhlm.

As part of some research on the naturally occurring substances of the South American flora[1], two of us (P.C.F., G.B.M.-B.) studied the alkaloids of *Aspidosperma longepetiolatum* Kuhlm. (Apocynaceae), known in Brazil by the name of *Guatambu amarelo*. About 1 kg of the bark was extracted with cold dilute acetic acid; the percolates were successively concentrated, the resulting acetic solution was adjusted to pH 8, and was then extracted first with ether and subsequently with chloroform. Evaporation of the solvents resulted in about 7 g of the raw alkaloids in form of their free bases. Both paper chromatography and electrophoresis evidenced the complexity of the mixture. Crystallization from ethanol yielded about 1 g of a compound (m.p. 232–238°) which was purified by chromatography over Al_2O_3; recrystallization from petroleum ether-benzene and from ethanol yielded prismatic crystals, m.p. 251° (decomp.), $[\alpha]_D^{25} = +106°$ (in pyridine); the compound was called *guatambuine*.

Direct comparison proved guatambuine to be identical with *u*-alkaloid C ($C_{18}H_{20}N_2$), one of the secondary carbazole alkaloids of *Aspidosperma ulei* Mgf. recently isolated by Schmutz and Hunziker[2] (m.p., optical rotation, UV- and IR-spectrum, colour reactions and Rf value).

While in *Aspidosperma ulei* Mgf. the *u*-alkaloid C represents one of the minor constituents, guatambuine can be considered one of the main constituents of the alkaloids of *Aspidosperma longepetiolatum* Kuhlm.

Aspidosperma longepetiolatum Kuhlm. contains three other indole alkaloids, the chromatographic separation of which is in progress.

We wish to express our appreciation to Prof. R. Wasicky (São Paulo) for having kindly supplied the required botanical drug.

P. Carvalho Ferreira, G. B. Marini-Bettolo, and J. Schmutz

Istituto Superiore di Sanità, Roma, and Research Institute, Dr. A. Wander S.A., Berne, February 9, 1959.

Riassunto

Viene descritto l'isolamento della guatambuina $C_{18}H_{20}N_2$ dalla corteccia dell'*Aspidosperma longepetiolatum* Kuhlm. (Apocynaceae), che costituisce uno degli alcaloidi principali della droga. La guatambuina si è rivelata identica all'u-alcaloide C, un derivato del carbazolo, precedentemente isolato in piccola quantità dalla corteccia dell'*Aspidosperma ulei* Mgf.

[1] G. B. Marini-Bettolo, Svensk farm. Tidskr. *59*, 25 (1955); Ann. chim. App. *38*, 305 (1948); Gazz. chim. Ital. *80*, 63 (1950).

[2] J. Schmutz and F. Hunziker, Helv. chim. Acta *41*, 288 (1958).

Beitrag zur Butazolidinwirkung auf Enzyme[1]

Zur Abklärung des Wirkungsmechanismus von Butazolidin[2] wurde von zahlreichen Autoren der Einfluss dieses Pharmakons auf fermentative Prozesse untersucht. In einer früheren Arbeit haben wir die bisher in der Literatur mitgeteilten Ergebnisse über diesen Fragenkomplex zusammengestellt (Stenger[3]). Im Hinblick auf die Bedeutung der ferment-chemischen Forschungsrichtung zur Wirkungsanalyse des Butazolidins halten wir es für berechtigt, unsere *in vitro*-Untersuchungen über die Monoaminoxydase und Cytochromoxydase mitzuteilen, auch wenn durch Butazolidin keine signifikante Inhibition dieser Fermente ausgelöst werden konnte.

Methodik. Die Messung des Sauerstoffverbrauches erfolgte im Warburg Modell S der Firma B. Braun, Melsungen. Reaktionsgefässe: ca. 15 ml; Gasraum: Luft; Versuchstemperatur: 37°C; Schüttelfrequenz: 100/min; Amplitude: 5 cm. Die Prüfsubstanz pipettierten wir in einer Menge von 0,2 ml in den Seitenarm, bei den Kontrollen 0,2 ml M/15 Sörensen Phosphatpuffer pH 7,4 direkt in den Hauptraum. Nach einem Temperaturausgleich von 15 min wurde die Prüfsubstanz eingekippt. 1 min später begann die Messung. Die Dosisangabe in mg% bezieht sich auf das Gesamtvolumen des Ansatzes.

Die Aktivität der Monoaminoxydase und Cytochromoxydase (Leber) wurde manometrisch bestimmt. Die Leber von jeweils 2–3 Ratten (männliche Tiere, 130 bis 160 g) verarbeiteten wir zu einem Homogenat.

Die Aktivität der Monoaminoxydase erfassten wir in Anlehnung an die von Robinson[4] und Spinks *et al.*[5] beschriebene Methode.

Ansatz: 0,2 ml KOH 20%ig + Filterpapier (Zentralzylinder)
1,0 ml Leberhomogenat (1 g Leber: 4 ml M/15 Phosphatpuffer pH 7,4)
1,0 ml M/15 Phosphatpuffer pH 7,4
0,2 ml Substrat (Tyramin 0,1 M; Serotonin 0,05 M)
0,3 ml KCN 0,1 M
0,2 ml Prüfsubstanz bzw. Puffer

Ein geeigneter Test zur Bestimmung der Cytochromoxydase-Aktivität beruht auf der Oxydation von *p*-Phenylendiamin (Battelli *et al.*[6]; Szent-Györgyi[7]). Die Leber wurde in einem feinen Tuch ausgepresst und nach Zusatz von destilliertem Wasser (1 g Leber: 10 ml Wasser) 1 min homogenisiert. Das Homogenat zeigte ohne Zusatz von *p*-Phenylendiamin praktisch keine Atmung.

Ansatz: 1,0 ml M/15 Sörensen Phosphatpuffer pH 7,4
1,3 ml Leberhomogenat
0,2 ml *p*-Phenylendiamin 1%ig (gelöst in M/15 Phosphatpuffer pH 7,4)
0,2 ml Prüfsubstanz bzw. Puffer

Resultate. Aus den Daten der nachfolgenden Tabelle ist zu ersehen, dass Butazolidin in einer Dosis von 10 mg% keine signifikante Beeinflussung der Monoamin- und Cytochromoxydase-Aktivität (Leber) auslöst. Der Befund, dass Butazolidin die Cytochromoxydase nicht blockiert, steht in Einklang mit den Ergebnissen, wonach Butazolidin in therapeutisch wirksamen Konzentrationen keine Hemmung der Atmungskette bewirkt (Bässler *et al.*[8]; Kersten *et al.*[9]). Iproniazid bzw. Kaliumcyanid bewirkten unter identischen Versuchsbedingungen in den untersuchten Dosen eine eindeutige Fermentinhibition.

E. G. Stenger

J. R. Geigy A.G., Basel, 5. März 1959.

Summary

Butazolidin in a concentration of 10 mg% does not inhibit *in vitro* the activity of rat liver monoamine- and cytochromoxydase.

[1] Die Untersuchungen wurden im Pharmakologischen Institut der Universität des Saarlandes, Homburg, durchgeführt.
[2] Geigy.
[3] E. G. Stenger, Dtsch. Med. J. *9*, 331 (1958).
[4] J. Robinson, Brit. J. Pharmacol. *7*, 99 (1952).
[5] A. Spinks und J. H. Burn, Brit. J. Pharmacol. *7*, 93 (1952).
[6] F. Battelli und L. Stern, Biochem. Z. *46*, 317, 343 (1912).
[7] A. Szent-Györgyi, Biochem. Z. *150*, 195 (1924).
[8] K. H. Bässler und K. Lang, Arch. exp. Path. Pharmakol. *229*, 568 (1956).
[9] H. Kersten und Hj. Staudinger, Z. Rheumaforsch. *16*, 259 (1957).

Tabelle

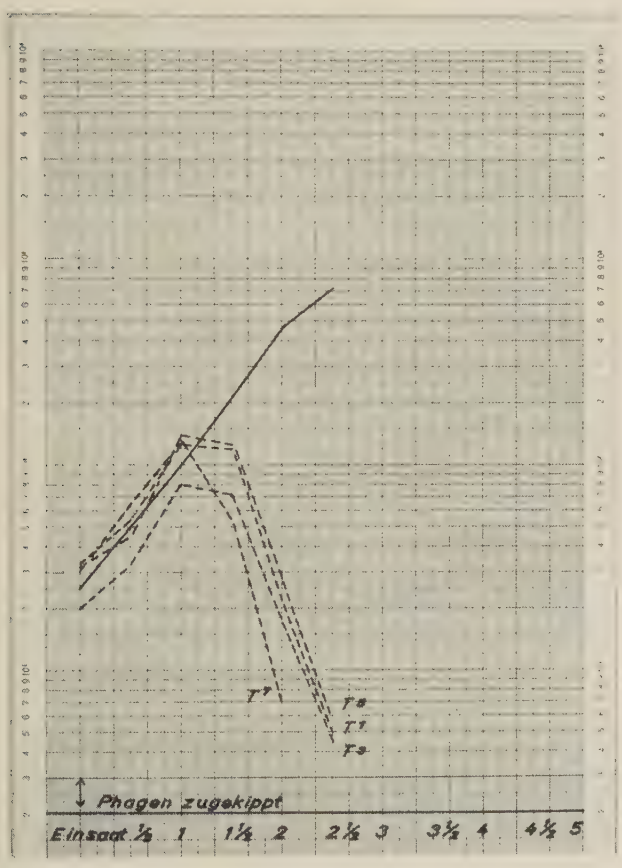
Aktivität der Monoamin- und Cytochromoxydase (Leber) nach Zusatz von Butazolidin[1]

Substrat	Prüfsubstanz	Dosis mg%	n[3]	O_2-Verbrauch in mm³ nach				Hemmung in % nach			
				30 min	60 min	90 min	120 min	30 min	60 min	90 min	120 min
A. *Monoaminoxydase*											
Tyramin 0,1 M . . .	Butazolidin	10	7	18/19[2]	30/30	41/41	47/48	5	0	0	2
Tyramin 0,1 M . . .	Iproniazid	5,55	28	6/16	11/25	14/32	17/38	63	56	56	55
Serotonin 0,05 M . .	Butazolidin	10	7	15/16	23/24	28/28	30/30	6	4	0	0
B. *Cytochromoxydase*				15 min	30 min	45 min		15 min	30 min	45 min	
p-Phenylendiamin 1%	Butazolidin	10	6	16/16	28/29	37/38		0	3	3	
p-Phenylendiamin 1%	Kaliumcyanid	0,02	6	12/16	21/29	28/38		25	28	26	

[1] ohne Lokalanaesthetikum
[2] . . /. . Substanz/Kontrollen (jeweils gleiche Zahl von Einzelbestimmungen)
[3] Zahl der Einzelbestimmungen

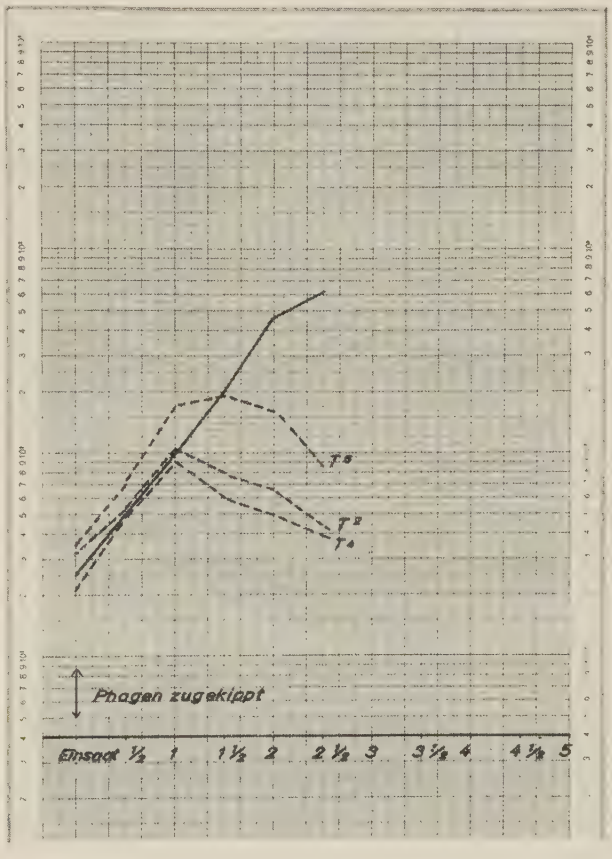
Comparaison de l'activité respiratoire d'*Esch. coli* pendant la bactériolyse due aux bactériophages de la série T_1 à T_7

Dans des communications précédentes[1], considérant la série *T* des bactériophages d'*Escherichia coli* Delbrückii, nous avons mis en évidence, *in vitro*, que des dérivés de la quinazoline inhibent les bactériophages T_1, T_3, T_5, T_7, mais ne semblent avoir aucune action vis-à-vis de T_2, T_4, T_6. Ayant utilisé précédemment la méthode de Warburg[2] afin de déterminer les variations de la consommation d'oxygène pendant la multiplication microbienne (en particulier, pendant la phase logarithmique), nous avons pensé qu'elle serait indiquée pour l'analyse plus exacte du processus de la lyse de bactériophages de types différents. Ce test touchant au métabolisme du complexe [bactérie-bactériophage] permettra peut-être de déceler des différences particulières.



Courbes 1. *E. coli* Delbrückii + phages T_1, T_3, T_5, T_7 en bouillon Difco 6%. *E. coli* Delbrückii (contrôle) ——, Bactériophages – – – –.

Méthode. Nous avons analysé le métabolisme bactérien (variation de la quantité d'oxygène utilisée) à l'aide de la méthode de Warburg. La souche d'*Escherichia coli* Delbrückii et la série *T* (de 1 à 7) des bactériophages ont été choisis comme tests. Dans le récipient se trouve la culture bactérienne en bouillon Difco à 6%. La suspension de bactériophages (de concentration déterminée: par exemple pour T_1: 0,2 ml d'une culture diluée à 10^{-6}) est placée dans le petit appendice latéral. La substance expérimentée est ajoutée à la concentration désirée, soit dans le récipient principal, soit dans un second appendice si l'on veut en étudier l'action au cours du développement du bactériophage. La quantité de bactéries, facteur important, doit être déterminée soigneusement au début de chaque essai: soit néphélométriquement, soit par le volume d'oxygène consommé pendant un temps déterminé. Le développement bactérien optimum correspond à une consommation d'oxygène d'environ 20 mm³ en 30 min lorsque l'équilibre de température à 37°C a été réalisé. On ajoute alors la suspension de bactériophages. La température est maintenue rigoureusement à 37°C pendant toute l'expérience, c'est-à-dire pendant 3 à 4 h. Des lectures manométriques du volume d'oxygène utilisé ont lieu toutes les 30 min.



Courbes 2. *E. coli* Delbrückii + phages T_2, T_4, T_6 en bouillon Difco 6%. *E. coli* Delbrückii (contrôle) ——, Bactériophages – –.– –.

Résultats. La courbe de l'intensité respiratoire ainsi que la courbe de croissance d'*Esch. coli* Delbrückii ont une allure très semblable à celles d'*Esch. coli* 207, souche expérimentée précédemment[2]. Il s'ensuit que ces deux souches doivent avoir un métabolisme très voisin.

Les essais effectués avec la série *T* des bactériophages ont montré le très intéressant phénomène suivant: les différents types *T* montrent un processus de lyse différent qui permet de les ordonner en deux groupes.

[1] R. Meier, L. Neipp et W. Kunz, Schw. med. Wschr. *87*, 1427 (1957). – L. Neipp, W. Kunz et R. Meier, Exper. *13*, 74 (1957).

[2] L. Neipp, W. Kunz et R. Meier, Schw. Z. Path. Bakt. *19*, 331 (1956).

Dans le premier groupe, les bactériophages T_1, T_3, T_5, T_7 provoquent la bactériolyse après un certain temps de latence qui dépend de divers facteurs (quantité de phages ensemencés, moment où cette addition a lieu, etc.). Cette lyse se traduit par une diminution rapide de la «consommation d'oxygène» pouvant aller jusqu'à la suppression presque totale de celle-ci; par suite, la courbe respiratoire fait une chute rapide qui aboutit presque à la valeur zéro. Cette courbe correspond également à celle d'*Esch. coli* 207 jusque dans ses moindres détails.

L'autre groupe qui comprend les bactériophages T_2, T_4, T_6 est caractérisé, après une phase de latence déterminée, par une bactériolyse qui s'effectue plus lentement. La «consommation d'oxygène» s'élève seulement jusqu'à une certaine valeur et y stationne longuement. Cette phase lytique régulière se traduit sur la courbe par un «plateau» qui peut être légèrement descendant; l'intensité respiratoire se maintient à un niveau déterminé ou diminue légèrement en dessous de celui-ci.

On peut en déduire que le dommage subi par les bactéries ou le nombre de celles qui sont atteintes par l'action phagique est différent dans les deux cas.

En outre, nous avons pu vérifier que la lyse produite avec le groupe $T_{2,4,6}$ quoique plus lente, est complète. Celle qui est déployée par $T_{1,3,5,7}$, d'abord beaucoup plus rapide, se révèle par la suite incomplète et montre l'apparition ultérieure d'individus résistants.

Conclusions. Le cours de la bactériolyse, suivi par la mesure du volume d'oxygène utilisé par les bactéries, est différent chez les bactériophages $T_{1,3,5,7}$ et $T_{2,4,6}$. Après un temps de latence, elle est rapide et incomplète avec $T_{1,3,5,7}$; elle est lente avec un «plateau» respiratoire et complète avec $T_{2,4,6}$. Le mécanisme biochimique de la bactériolyse semble donc différent dans les deux cas.

L. Neipp, W. Kunz et R. Meier

Laboratoire de Recherche du Département pharmaceutique de CIBA Société Anonyme Bâle, le 2 mars 1959.

Summary

The course of bacteriolysis as determinated by the oxygen consumption of the bacteria is different during bacteriolysis produced by the phages $T_{1,3,5,7}$ as compared with that produced by phages $T_{2,4,6}$. With phages $T_{1,3,5,7}$, the bacteriolysis occurs, rapidly, but incompletely after a latent period. With the phages $T_{2,4,6}$ bacteriolysis occurs slowly and the course of the respiration shows a «plateau» but goes to completion. The biochemical mechanism of the bacteriolysis therefore seems to differ in both cases.

Increase of the Cathepsin Activity of the Liver and of the Skeletal Muscle of Rats treated either with 2,4-Dinitrophenol or with Bacterial Lipopolysaccharide

It is known that protein catabolism is strongly accelerated during fever, as is shown by the increased urinary elimination of nitrogen, especially in the form of ammonia, uric acid, and creatine (Grafe[1], Colemann, and Dubois[2], Shafter and Colemann[3]). The reasons for this increased catabolism are not completely known.

The present paper deals with the behaviour of cathepsin activity of both liver and skeletal muscle of rats injected with two pyrogenic substances, i. e. either with 2,4-dinitrophenol (DNP) or with the lipopolysaccharide of *Salmonella abortus equi* (LPS)[4]. DNP, when injected intraperitoneally in doses of 4 mg/100 g body weight, produces a rise of body temperature of about 1°C within 1 h. LPS does not constantly increase the body temperature of rats, but in some instances it produces hypothermia. When the amount of LPS injected intraperitoneally in the rat was 0·25 µg/100 g body weight, an increase of 0·7–1°C of rectal temperature was recorded in about 70% of the cases studied within 1 h. In the present investigation, only the rats which developed fever were studied.

Albino rats weighing 160–180 g were used. They were fed on a standard diet and their body temperature was recorded for many days before the beginning of the treatment. The animals were killed by decapitation 2 h after the injection. Body temperature was measured before the injection and before the death. The liver or the muscle of the right leg were immediately dissected and transferred to the cold room at + 2°C.

Different groups of animals were used to study the cathepsin activity of the liver and of the skeletal muscle, in order to decrease the time necessary for the preparation of the homogenates. 10% homogenates were prepared in the Potter-Elvehjem homogenizer. Skeletal muscle was previously finely minced with scissors.

In each case two types of suspension fluids were used: the first one was 0·25 *M* sucrose, the second one was 0·25 *M* sucrose containing 0·1% Triton X-100. This was used to produce maximal activation of cathepsin as a result of the disintegration of the particles (lysosomes) in which it is contained (Wattiaux and DeDuve[5]).

Cathepsin activity was determined at 37°C by the method of Gianetto and DeDuve[6], with 0·17 *M* acetate buffer, pH 5·0, and 0·00026 *M* hemoglobin as substrate. In the case of the homogenates prepared with Triton X-100, also the reaction fluid contained this substance in 0·1% concentration. The reaction was stopped by addition of ice cold 0·3 *M* trichloroacetic acid. Three different amounts of enzyme material were used for each determination: 20, 50, and 100 mg. In each case, two samples were used: in the first one the reaction was stopped 2 min after the beginning of the incubation; in the second one it was stopped after 10 min. The aromatic degradation products of hemoglobin were then determined on the deproteinized filtrate, with tyrosine as standard. The differences between the readings at 10 and at 2 min were retained as a measure of cathepsin activity. The values obtained were submitted to statistical analysis, the standard deviation being calculated for each average and the '*t*' value of Fisher for each difference between two averages. Only the data with a '*t*' value corresponding to a probability $P < 0{\cdot}05$ were accepted as significant.

Table I shows that in the rats treated either with DNP or with LPS, the cathepsin activity of liver homogenates

[1] E. Grafe, *Die Wärmeregulation und ihre Störungen*, Oppenheimers Handbuch der Biochemie, Ergänzungswerk, vol. 1.

[2] E. Du Bois, J. Amer. med. Ass. *77*, 35 (1920).

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[4] The author is indebted to the Firma Wander S.A., Berne, for generous supply of LPS (Pyrexal).

[5] R. Wattiaux and C. DeDuve, Biochem. J. *63*, 606 (1956).

[6] R. Gianetto and C. DeDuve, Biochem. J. *59*, 433 (1955).

Table I. Behaviour of cathepsin activity of the rat liver after treatment either with 2,4-dinitrophenol (DNP) or with the lipopolysaccharide of *Salmonella abortus equi* (LPS)

The standard deviation is given after each average

	Amount of the enzyme used mg	Normal rats (15 experiments)		Rats treated with DNP (9 experiments)		Rats treated with LPS (8 experiments)	
		μg tyrosine set free in 8 min	μg tyrosine mg N	μg tyrosine set free in 8 min	μg tyrosine mg N	μg tyrosine set free in 8 min	μg tyrosine mg N
Without Triton	20	0	0	0	0	14·4 ± 5·3	26·3 ± 7·8
With Triton	20	32·7 ± 9·6	57·5 ± 32·3	38·5 ± 13.3	68·8 ± 23.7	54·7 ± 14·7	99·3 ± 28·6
Stimulation by Triton %		∞				279·8 +	
Without Triton	50	7·3 ± 7·0	5·1 ± 5·0	17·7 ± 12·0	12·5 ± 8·8	26·1 ± 8·0	19·5 ± 6·2
With Triton	50	62·4 ± 18.3	44·1 ± 16.4	47·3 ± 19·3	33·7 ± 14·9	68·6 ± 24.8	51·0 ± 19·4
Stimulation % by Triton		+754·8		+167·7		+162·8	
Without Triton	100	14·3 ± 9.6	5·1 ± 3.1	46·0 ± 20·7	16·6 ± 8·4	32·0 ± 12·9	11·9 ± 5·1
With Triton	100	99·7 ± 18·8	34·0 ± 7·8	76·1 ± 28·5	24·9 ± 9·8	87·8 ± 24.0	32·7 ± 9·3
Stimulation % by Triton		+583·2		+65·2		+174·3	

prepared without Triton is strongly increased. No significant differences were noted with homogenates prepared in the presence of Triton X-100. Table II shows that also cathepsin activity of skeletal muscle is strongly increased in treated rats. In this case, however, some differences existed between the animals treated with DNP and those treated with LPS. In fact, in the latter group the cathepsin activity was found to be increased not only in the experiments made in the absence of Triton, but also in those made in the presence of this substance. The extent of the increase was higher in animals treated with LPS than in those treated with DNP.

Since the cathepsin which acts on hemoglobin is located within lysosomes, the fact that the enzyme activity of the liver of treated animals increases in the absence of Triton but remains unmodified in the presence of this substance, suggests that the change is not due to a real increase of the amount of the enzyme contained in the tissue, but to a damage of the particles in which it is contained. In the case of the skeletal muscle of rats treated with LPS, in which the increase was observed also in the presence of Triton X-100, it seems probable that an increase of the amount of the enzyme, or also a different type of activation, play an important role.

The increase of cathepsin activity in the organs of animals treated with pyrogenic substances may have some importance in understanding the mechanism of the accelerated protein catabolism found by many authors during fever.

E. Martini

Institute of General Pathology of the University of Genoa (Italy), December 9, 1958.

Riassunto

L'attività cateptica dell'omogenato aumenta notevolmente nel fegato e nel muscolo scheletrico di ratti trattati con 2,4-dinitrofenolo, oppure col lipopolisaccaride della *Salmonella abortus equi*, due sostanze piretogene. L'aumento è evidente in animali uccisi 2 h dopo l'iniezione, in fase febbrile. Esso si osserva solo con gli omogenati preparati senza Triton X-100, una sostanza tensioattiva che disintegra i lisosomi, organelli nei quali la catepsina è contenuta. Nessun aumento si osserva, nel caso del fegato, in presenza di Triton. Nel caso del muscolo scheletrico, invece, l'attività cateptica è aumentata anche in presenza di Triton, specialmente negli animali trattati col lipopolisaccaride.

Table II. Behaviour of cathepsin activity of the rat skeletal muscle after treatment either with 2,4-dinitrophenol (DNP) or with the lipopolysaccharide of *S. abortus equi* (LPS)

The standard deviation is given after each average

	Amount of the enzyme used mg	Normal rats (9 experiments)		Rats treated with DNP (8 experiments)		Rats treated with LPS (15 experiments)	
		μg tyrosine set free in 8 min	μg tyrosine mg N	μg tyrosine set free in 8 min	μg tyrosine mg μg	μg tyrosine set free in 8 min	μg tyrosine mg N
Without Triton	20	0	0	7·9 ± 7·7	18·7 ± 18·3	22·6 ± 7·5	50·2 ± 19·1
With Triton	20	22·7 ± 7·1	47·9 ± 16·2	18·3 ± 7·4	43·6 ± 18·2	55·6 ± 12·7	118·1 ± 30·2
Stimulation by Triton %		∞		+131·6		+146·0	
Without Triton	50	13·9 ± 12·8	11·1 ± 8·7	32·7 ± 21·2	29·2 ± 18·1	69·4 ± 18·9	53·0 ± 15·4
With Triton	50	31·2 ± 10·5	24·8 ± 9·8	41·5 ± 18.8	37·8 ± 16·9	115·7 ± 33·3	100·2 ± 35·4
Stimulation % by Triton		+124·4		+ 26·9		+ 65·1	
Without Triton	100	20·7 ± 7·5	8·3 ± 3·5	91·2 ± 30·9	38·8 ± 11·9	79·3 ± 28·4	33·7 ± 12.8
With Triton	100	85·9 ± 13·1	34·9 ± 6·3	96·7 ± 16·7	41·5 ± 6·5	148·9 ± 40·9	63·8 ± 17·1
Stimulation % by Triton		+314·9		+6·0		+87·7	

The Cucumber Tendril – a New Test Organ for Gibberellic Acid

There is an evergrowing interest in gibberellic acid (GA) and other gibberellins, both for their possible practical application in agriculture[1], and as an aid in the solution of theoretical problems of growth and flowering in higher plants.

As a part of an investigation of the genetics and physiology of the cucumber[2], started at the Plant Genetics Section of the Weizmann Institute of Science, it was intended to study the role of GA. Thus, a simple method for the estimation of gibberellic acid-like substances in plant extracts was looked for.

The possibility of using cucumber tendrils as test organs for GA, became apparent in an experiment in which the tendrils of young, GA-treated, plants appeared several days earlier than those of control plants. It was first found that 10 mm sections of straight tendrils, when incubated for about 24 h in solutions of 1·0 ppm. GA, 50 ppm. GA, 1·0 ppm. indoleacetic acid (IAA), 50 ppm. IAA, and in control solution, had a final length of $14{\cdot}7 \pm 0{\cdot}3$, $15{\cdot}0 \pm 0{\cdot}3$, $14{\cdot}0 \pm 0{\cdot}2$, $14{\cdot}5 \pm 0{\cdot}2$ and $13{\cdot}9 \pm 0{\cdot}3$ mm respectively. Here it is of particular interest that GA and IAA immersed tendril sections differed more in their form than in their length. While those incubated in GA were still straight or somewhat bent, those incubated in IAA were twisted (see Fig. 1). Similar twisting was observed with indolebutyric acid and indolepropionic acid.

The effect of incubation period was further tested. Fifteen 10 mm sections were incubated for 6, 21 and 44 h in small glass vials containing control, 1 ppm. and 10 ppm. GA solutions. Results presented in table form show only slight elongation after 6 h, but significant increase in length over the control as effected by the 1·0 and 10·0 ppm. GA solutions, after 21 h. Although still further elongation can be detected after 44 h, the difference between the 1·0 and 10·0 ppm. solutions as observed in this and other tests seem to decrease, when the incubation period is much more than 24 h.

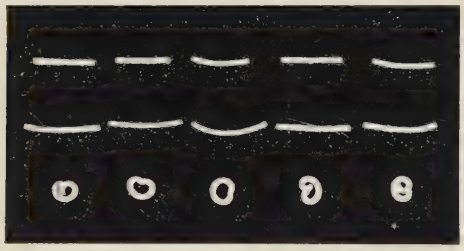

Fig. 1.—Three groups of five 10 mm cucumber tendril sections, incubated in different solutions: upper group – control; middle group – 1·0 ppm. GA solution; and lower group – 1·0 ppm. IAA solution.

In another test the reaction of different regions along the tendril to various concentrations of GA was investigated. Sections from tendril regions 25–35, 35–45, 45–55, and 55–60 mm from the tip were incubated in control, 0·33 ppm., 1·0 ppm., 3·3 ppm. and 10·0 ppm. GA solutions for 24 h. Each tendril region and each concentration was

Elongation of 10·0 mm sections of cucumber tendrils incubated for 6, 21 and 44 h in control, 1·0 ppm. and 10·0 ppm. GA solutions. Numbers indicate mm final length.

GA concentrations	Incubation time in h		
	6	21	44
Control	$11{\cdot}7 \pm 0{\cdot}14$	$12{\cdot}4 \pm 0{\cdot}20$	$12{\cdot}9 \pm 0{\cdot}15$
1·0 ppm. GA. .	$11{\cdot}7 \pm 0{\cdot}08$	$13{\cdot}3 \pm 0{\cdot}23$	$14{\cdot}7 \pm 0{\cdot}10$
10·0 ppm. GA .	$12{\cdot}0 \pm 0{\cdot}14$	$14{\cdot}0 \pm 0{\cdot}33$	$14{\cdot}9 \pm 0{\cdot}48$

tested by 15 (tendril region 25–35 mm from the tip) or 20 (all other regions) sections. Results and LSD values for each tendril region are presented in Figure 2.

It can be seen that as the distance from the tip increases the elongation decreases; moreover, the elongation ability of the '55–60 mm' tendril region is too small to serve as tester for GA. The optimum of elongation, for the three other regions, is near to 3·3 ppm. and a continuous increase of elongation from 0·33 ppm. to 3·3 ppm. GA as well as a decrease from 3·3 to 10·0 ppm. GA is apparent.

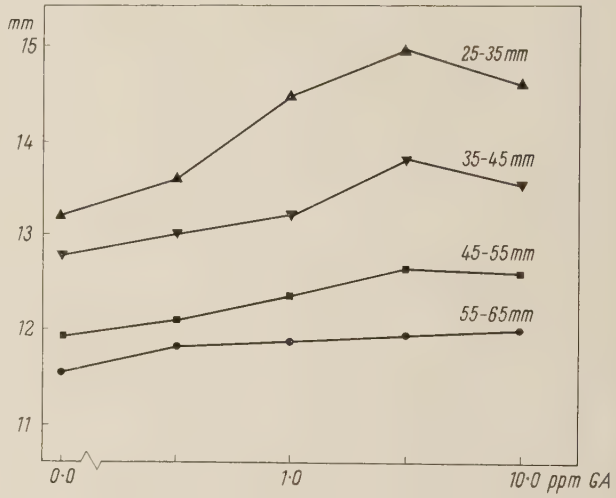

Fig. 2.—The effect of GA concentration on 10·0 mm sections taken from various regions along the tendril. Results are expressed in mm final length. The LSD values for the 25—35 mm, 35—45 mm, 45—55 mm, and 55—60 mm regions were 0·51 mm, 0·43 mm, 0·28 mm, and 0·35 mm respectively.

Based both on the data presented above and on those of other similar tests, the following method was adopted. Tendrils were harvested from greenhouse-grown cucumber plants of inbred lines (*Beit Alpha*, selfed for seven generations). A daily crop of about 25 straight tendrils, 13–14 cm long, was received from every 100 plants. Tendrils can be harvested from the fourth to the eighth week after emergence. From every tendril 10 mm sections of two regions (25–35 and 35–45 mm from the tip) were cut. Five sections of the same region were put in every 55×15 mm glass vial. The glass vials contained 1·0 ml of a buffer-sucrose solution (1·019 g citric mono hydrate, 1·749 g Potassium phosphate and 30·0 g sucrose/l) and a 50×15 mm piece of filter paper to which a certain amount of GA was applied by means of a micropipette, or which was cut out after paper chromatographic separation. Every amount of GA was tested with five sections of each of the two regions and the test was repeated at least once.

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Comparing this test to others recently suggested, some advantages of the present method seem to be apparent. PHINNEY *et al.*[3] suggested maize dwarf mutants as test plants; the tested material is to be applied by means of drops on intact leaves and results 'read' after several days. With this method, as well as with the cucumber leaf and the dwarf pea seedlings methods[4], the paper strips, representing different *Rf* values, after chromatographic separation, cannot be tested directly but must be eluted and reconcentrated before application. The wheat leaf sections test of RADLEY[5] can be used directly with filter paper strips but only qualitative values are obtained. Since the cucumber tendril test has a simple procedure, gives fair quantitative estimates of GA and can be combined directly with paper chromatographic separation of plant extracts, its further use is being advocated.

The author is indebted to Miss R. SMILANSKI-DROR for her skillful technical help. The GA sample was donated by Dr. P. W. BRIAN of the Imperial Chemical Industries, England.

E. GALUN

Plant Genetics Section, Weizmann Institute of Science, Rehovoth (Israel), December 29, 1958.

Résumé

On peut se servir de tronçons de vrilles de concombre en voie de dévelopment *in vitro*, pour déterminer d'après leurs réactions la présence d'acide gibberellique (GA). L'avantage de cette méthode réside dans le fait qu'on peut l'employer en la combinant avec la chromatographie sur papier et ainsi évaluer le GA dans l'extrait des plantes.

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Vobasin und Voacryptin, zwei neue Alkaloide aus *Voacanga africana* Stapf

Kürzlich isolierte RAO[1] aus der Apocynacee *Voacanga africana* Stapf die Alkaloide Voacafrin und Voacafricin, die mit einer spektralen Absorption bei 240 und 315 mμ gegenüber den bis jetzt bekannten Voacanga-Alkaloiden Voacangin[2,3], Voacamin[3,4], Vobtusin[4], Voacorin[5], Voacamidin[6], Voacristin[6] und Voacangarin[7] einen neuen Spektraltyp repräsentieren. Diese Arbeit veranlasst uns zu der Mitteilung, dass wir aus *Voacanga africana* Stapf ebenfalls ein neues Alkaloid dieses Spektraltyps isoliert haben, für das wir den Namen Vobasin vorschlagen. Zu seiner Isolierung chromatographierten wir eine aus Stammrinde in üblicher Weise gewonnene Gesamtalkaloidfraktion an Aluminiumoxyd, trennten aus den Voacorin-Fraktionen dieses Alkaloid ab und unterwarfen die in den Mutterlaugen enthaltenen Basen nach Abtrennung schwerlöslicher Hydrobromide einer 24-stufigen Craig-Verteilung zwischen Citronensäure-Phosphatpuffer vom pH 4,8 und Benzol/Äther 1:1. Aus den Fraktionen 5–14 der Verteilung kristallisierte Vobasin nach Anreiben mit Äther; die Ausbeute betrug 0,07–0,1%.

Vobasin $C_{21}H_{24}O_3N_2$ (ber. C 71,57; H 6,86; N 7,95; gef. C 71,26; H 6,90; N 7,88), aus Äther derbe Würfel, Smp. 111–113°C; $[\alpha]_D^{23}$-158,5° ($CHCl_3$, c = 1); −148,3° (MeOH, c = 1), enthält je eine OCH_3– (ber. OCH_3, 8,80; gef. 9,06), eine N–CH_3– (ber. CH_3 4,26; gef. 4,39) und eine C–CH_3-Gruppe (ber. CH_3 4,26; gef. 3,48). Bei der Mikrohydrierung mit PtO_2 in Äthanol wurden 2 Mol H_2 verbraucht. Das UV-Spektrum (λ_{max} 239,5 mμ; log ε 4,19 und 315 mμ, log ε 4,27) entspricht dem des 2-Phenylindols[8]. IR-Spektrum in CH_2Cl_2: Banden bei 2,91 (NH); 3,60 (N–CH_3?); 5,79 (C=O, Ester) und 6,07 μ; in Nujol: starke Doppelbande bei 13,45 und 13,58 μ (1,2-disubst. Phenyl).

Vobasin-Hydrochlorid $C_{21}H_{25}O_3N_2Cl$ (ber. C 64,84; H 6,48; N 7,21; Cl 9,12; gef. C 64,41; H 6,49; N 7,28; Cl 9,07): aus Methanol prismatische Blättchen, Smp. 245 bis 248°C (Zers.); $[\alpha]_D^{22}$–120,1° (MeOH, c = 1).

Vobasin-Methojodid $C_{22}H_{27}O_3N_2J$ (ber. C 53,45; H 5,51; N 5,67; gef. C 53,31; H 5,58; N 5,71): aus Methanol prismatische Stäbchen, Smp. 212–214°C (Zers.); $[\alpha]_D^{23}$–117,2° (MeOH, c = 1).

Die physikalischen Daten der Base und ihres Hydrochlorids schliessen eine Identität mit Voacafrin oder Voacafricin aus.

Die im Vobasin enthaltene OCH_3-Gruppe ist Teil einer Methylestergruppierung. Alkalische Verseifung führte zu einer Aminosäure $C_{20}H_{22}O_3N_2$ (ber. C. 70,98; H 6,55; N 8,28; gef. C 70,94; H 6,59; N 8,43), Smp. 290–292°C (Zers.), die mit Diazomethan *Isovobasin*, $C_{21}H_{24}O_3N_2$ (ber. C 71,57; H 6,86; N 7,95; OCH_3 (1) 8,80; gef. C 71,40; H 6,86; N 8,05; OCH_3 8,37); Smp. 175 178°C (Zers.); $[\alpha]_D^{22}$ − 191,3° ($CHCl_3$, c = 1), lieferte. Gewisse Parallelen zwischen Vobasin und der stärker basischen Molekülhälfte des Voacamins (N–CH_3-Gruppe[9], Umlagerung bei alkalischer Verseifung[10]) lassen vermuten, dass Vobasin einer monomeren biogenetischen Vorstufe des dimeren Voacamins nahesteht.

Ein weiteres Nebenalkaloid, für das wir den Namen *Voacryptin* vorschlagen, wurde nach 24stufiger Craig-Verteilung von Voacamin-Mutterlaugen zwischen Citronensäure-Phosphatpuffer vom pH 4,2 und Benzol/Äther 1:1 aus den Fraktionen 20–24 isoliert: aus Äther lange, verfilzende Nadeln, Smp. 175–176°C; $[\alpha]_D^{22}$ + 24,7° ($CHCl_3$, c = 1). Das UV-Spektrum (λ_{max} 224 mμ, log ε 4,46 und 285 mμ, log ε 3,98) entspricht dem des Voacangins. Summenformel ($C_{22}H_{26}O_4N_2$ ber. C 69,09, H 6,85; N 7,33; OCH_3 (2) 16,22; C–CH_3 (1) 3,93; gef. C 68,83; H 6,91; N 7,63; OCH_3 16,22; C–CH_3 3,21) und IR-Spektrum in CH_2Cl_2 (Banden bei 5,80 [C = O, Ester] und 5,83 μ [C = O, Keton]) weisen auf die Struktur eines Oxovoacangins.

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[10] U. RENNER, unveröffentlicht.

Die Interpretation der Spektren verdanken wir Herrn Dr. E. Giród, die Mikroanalysen unserem Mikroanalytischen Laboratorium (Leitung Dr. H. Wagner).

U. Renner

Wissenschaftliche Laboratorien der J. R. Geigy A.G., Basel, den 21. Januar 1959.

Summary

Vobasine, a monomeric indole alkaloid of novel spectral type, and voacryptine, a monomeric 5-methoxyindole derivative, have been isolated from the bark of *Voacanga africana* Stapf.

'Active Ammonia'

In the course of our investigations on the possible active forms of ammonia, we studied the amination of α-keto acids by rat liver preparations. Liver from freshly sacrificed rats was washed with saline, minced and homogenized in saline with a Potter-Elvehjem type homogenizer. The incubation was carried out in 0·1 M pH 7·0 phosphate buffer. Each vessel received 20 μmol. of sodium pyrophosphate and 5 μmol. of malonic acid. The following additions were made at the level of twenty μmol. of each: adenosine triphosphate (ATP), ammonium phosphate, α-keto glutaric acid (or pyruvic acid) and adenosine phosphoramide (AMP-NH_2). The total volume was 3·2 ml. At the end of 2–3 h at 38°C trichloroacetic acid was added to a final concentration of 5%. The incubates were centrifuged and analyzed for amino acid by the ninhydrin method. The keto acid left was estimated by the method of Friedemann and Haugen[1]. The results are presented in Table I.

Table I
Amino acid formation in rat liver homogenates

No	Additions	Keto acid disappeared μmol	Amino acid formed μmol
1	None (tissue alone) . . .	None added	3·90
2	α-ketoglutaric acid (K.G.)	0·0	5·10
3	K.G plus ammonium phosphate	0·0	11·85
4	K.G plus ammonia plus ATP	20·0	28·65
5	K.G plus ATP	17·82	23·65
6	K.G plus AMP-NH_2 . . .	20·0	24·45

The results presented in Table II were obtained by elimination of each component from a complete medium consisting of liver homogenate, phosphate buffer, α-keto glutaric acid, pyrophosphate, malonic acid and adenosine phosphoramide. Using pyruvic acid in the place of α-keto glutaric acid, similar results were obtained.

[1] T. E. Friedemann and G. E. Haugen, J. biol. Chem. *147*, 415 (1943).

Table II
Relative importance of the components of the medium

Medium of Incubation	Keto acid disappeared μmol	Amino acid formed μmol
Complete medium	20·0	25·6
Minus malonic acid	19·67	32·7
Minus pyrophosphate . . .	19·67	26·8
Minus α-keto glutaric acid .	****	10·8
Minus liver homogenate . .	0·0	0·0
Minus AMP-NH_2	10·35	13·4

These data show that adenosine phosphoramide was as effective as a mixture of ATP and ammonia. ATP alone was quite active and this might be due to the endogenous formation of ammonia in the liver homogenate. Further experiments are in progress to test whether the role of AMP-NH_2 is confined to amino acid synthesis alone or is important in other amination (e.g. biosynthesis of glucosamine) and amidation (e.g. glutamine biosynthesis) reactions also. Further experimental details will be reported elsewhere. While these experiments were in progress, similar results were reported using preparations of *Mycobacterium*[2].

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Department of Chemistry, Adelphi College, Garden City, N.Y., and Biochemical Research Laboratory, Children's Unit, Creedmoor State Hospital, Queens Village, New York, January 6, 1959.

Zusammenfassung

Es wurde gefunden, dass Adenosinophosphoamidat (Na) die Synthese von Keto-Säuren aus Rattenleberhomogenaten anregt. Diese Verbindung kommt in ihrer Wirkung einer Mischung von Adenosintriphosphat und Ammonium gleich. Wir beabsichtigen, die Amino- und die Amido-Reaktionen der Reinverbindungen zu untersuchen, um festzustellen, ob die physiologisch wirksame Form des Ammoniums aus Adenosinphosphoamidat besteht.

[2] N. Katunuma, Arch. Biochem. Biophys. *76*, 547 (1958).

The Effect of Substance P on the Amount of 5-Hydroxytryptamine in the Gut

It has been shown in a previous work that substance *P* restored peristalsis when injected intraluminally into the isolated guinea-pig ileum in which the peristaltic reflex was abolished by fatigue, by external or internal application of 5-hydroxytryptamine, or by lowering the temperature of the bath[1]. On the other hand, substance *P* when acting on the outside of the isolated guinea-pig ileum produced a block of the peristaltic reflex[2]. 5-Hydroxytrypt-

[1] D. Beleslin and V. Varagić, Brit. J. Pharmacol. *13*, 321 (1958).
[2] D. Beleslin and V. Varagić, J. Pharm. Pharmacol. *11*, 99 (1959).

Table I
The effect of substance P on the amount of 5-HT in the ileum, stomach and spleen of the rat

Number of experiments	ILEUM (ng 5-HT/g)		P	STOMACH (ng 5-HT/g)		P	SPLEEN (ng 5-HT/g)		P
	Controls	Substance P 100 U/kg		Controls	Substance P 100 U/kg		Controls	Substance P 100 U/kg	
1	1130	1574		1126	2086		1871	2307	
2	1303	2742		1598	1860		1000	1498	
3	1215	1670		1056	1452		714	1918	
4	754	1336		1553	1558		857	1428	
5	720	2218		988	1684		1124	615	
6	826	1578		891	2072		1076	1198	
7	340	1800		1919	2374		1810	1995	
8	902	1460		711	1743		1082	1018	
9	1480	1572		951	2198		972	1047	
10				1694	2308		705	1869	
Mean ± St. Error	964 ± 118·1	1773 ± 146·1	< 0·01	1249 ± 119·9	1933 ± 96·2	< 0·01	1121 ± 128·4	1489 ± 191·8	< 0·1

amine affected peristalsis in a similar way, i.e. it stimulated peristalsis when acting on the mucous membrane of the intestine[3] and it depressed or abolished peristaltic reflex when acting on the outside of the isolated intestine[4,5]. It was suggested in a previous work that substance *P* may act through another active substance[1]. Considering the similarity with which substance *P* and 5-HT act on the peristaltic reflex, the present experiments were undertaken in order to see whether substance *P* can alter the 5-HT content of the gut.

In the isolated guinea-pig ileum of 15–20 cm long, the substance *P* (60–80 units) was injected intraluminally and was allowed to act for 20–35 min. The control preparation was injected with saline. Both control and test preparation were prepared according to a modified Trendelenburg's technique which is described in detail in a previous paper[1]. The substance *P* was then washed out and the ileum was extracted with 80% acetone according to the method of CORREALE[6]. The extracts were assayed on the isolated rat fundus[7] in the presence of atropine (2×10^{-8} g/ml) and antazoline (10^{-6} g/ml).

In another series of experiments on rats, substance *P* was injected intraperitoneally in doses ranging from 60–120 units/kg. The injections of substance *P* were repeated in three successive days and on the fourth day the animals were sacrificed. From each animal the stomach, the spleen and a piece of ileum 20 cm long were taken and extracted with aceton. The extracts were assayed against 5-hydroxytryptamine on the isolated rat fundus in the presence of atropine (2×10^{-8} g/ml) and antazoline (10^{-6} g/ml).

The results are presented in the Tables I and II.

It may be seen that substance *P* increased the amount of 5-hydroxytryptamine both in the isolated guinea-pig ileum and in the rat ileum. The same was true for the rat stomach. On the other hand, no change in the 5-hydroxytryptamine content of the rat spleen was observed.

Table II
The effect of substance P on the amount of 5-HT in the isolated guinea-pig ileum

Number of experiments	Controls (ng 5-HT/g)	Substance P 60–80 Units Intraluminally (ng 5-HT/g)	P
1	350	640	
2	627	928	
3	551	1064	
4	339	604	
5	641	961	
6	414	686	
7	665	830	
8	467	691	
9	417	2618	
10	562	1115	
Mean ± St. Error	503 ± 35·1	1014 ± 154·2	< 0·01

STACEY and SULLIVAN[8] have found that an increase in the tryptophan content of the diet of mice and rats resulted in a rise in the amount of 5-HT in the small intestine. Sterilization of the gut by streptomycin and chlortetracycline had a similar effect, probably by preventing bacterial metabolism of dietary tryptophan. The present experiments show that intraluminal or intraperitoneal injection of substance *P* may also increase the amount of 5-hydroxytryptamine in the stomach and in the ileum. The exact mechanism of this effect of substance *P* cannot be accounted for by the present experiments.

5-hydroxytryptamine used in this work was kindly supplied by Farmitalia, Milano, Italy.

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Department of Pharmacology, Medical Faculty, Belgrade (Yugoslavia), March 4, 1958.

Résumé

L'injection intrapéritonéale de la substance *P* (60 à 120 U/kg) chez le rat provoque une augmentation mar-

[3] E. BÜLBRING and R. C. Y. LIN, J. Physiol. *138*, 12 P (1957).
[4] H. W. KOSTERLITZ and J. A. ROBINSON, J. Physiol. *136*, 249 (1957).
[5] K. H. GINZEL, J. Physiol. *137*, 62 P (1957).
[6] P. CORREALE, J. Neurochemistry *2*, 201 (1958).
[7] J. R. VANE, Brit. J. Pharmacol. *12*, 334 (1957).
[8] R. S. STACEY and T. J. SULLIVAN, J. Physiol. *137*, 63 P (1957).

quée de 5-HT dans la paroi de l'estomac et dans l'iléon. Dans la rate de cet animal, on n'y observe pas d'augmentation de 5-HT.

L'administration intraluminale de la substance *P* (60–80 U) dans l'iléon isolé du cobaye, augmente aussi sensiblement la quantité de 5-HT.

On the Excretion of 17-Ketosteroids in Guinea Pigs

Several investigations have revealed that the adrenocortical activity in guinea pigs is relatively high. They therefore excrete a number of 17-ketosteroids (17-KS) in the urine. Peron *et al.*[1] isolated the following 17-KS from *male* guinea pig urine:

3α-hydroxyetiocholan-17-one, 3α-hydroxy-$\triangle^{9(11)}$-etiocholen-17-one, 3α-hydroxyetiocholan-11,17-dione, 3α-,11β-dihydroxyetiocholan-17-one,3α-hydroxy-$\triangle^{9(11)}$-androsten-17-one, 3α-hydroxyandrostan-11,17-dione, 3α,11β-dihydroxyandrostan-17-one.

They were all 3α-compounds, of which the final identification was made by infrared analysis.

In our experiments, which will be reported in detail elsewhere, we isolated a *3β-hydroxy-17-ketosteroid* from the urine of normal untreated *female* guinea pigs. This has not previously been reported, under similar experimental conditions. Moreover, we confirmed the findings of Peron *et al.*[1].

The urine of 10 female guinea pigs was collected daily for 6 days. After β-glucuronidase hydrolysis and subsequent cold acid hydrolysis at pH 0·5 with continuous ether extraction (for 48 h), the 17-KS containing extracts were chromatographed on alumina by gradient elution technique[2]. We obtained 7–8 Zimmermann-positive fractions. As is shown in Table I, fraction II was the greatest. It amounted to 44·9% of the total Zimmermann chromogens eluted and contained a 17-KS, which showed the same running rate as crystalline *epiandrosterone* on the paper chromatogram (chromatographic system used: propylene glycol/ligroin). In the mixed chromatogram of both the isolated substance and pure epiandrosterone, no separation occurred.

The sulphuric acid spectrum of the unknown revealed maximal optical density at 310 mμ. No increased absorption was apparent at 405 mμ, which is characteristic for dehydroepiandrosterone. The paper chromatographic properties of epiandrosterone and dehydroepiandrosterone are not much different. Both steroids have almost the same running rate in the system propylene glycol/ligroin.

The 3β-configuration of the isolated steroid was demonstrated by digitonin precipitation of the purified fraction II. Less than 10% of it appeared in the α-fraction, while the bulk of Zimmermann positive material was measured in the β-fraction. The infrared spectrum of the β-fraction was identical with that of crystalline epiandrosterone. The described substance could be isolated repeatedly in different series of experiments.

The question as to the nature of the 17-KS conjugates appearing in the urine of guinea pigs seemed to be of particular interest. In order to investigate the mode of conjugation of 17-KS in guinea pigs, we followed the procedure of Crepy *et al.*[3] given for the extraction and separation of steroid conjugates in human urine.

A 6-day-urine specimen collected from 10 female guinea pigs was adjusted to pH 11, followed by the addition of sodium chloride until a saturation of approximately 10% was achieved. The urine was then extracted twice with the same volume of n-butanol. The extract so obtained was chromatographed on alumina as reported by Crepy *et al.*[3]. In this manner it was separated into 3 Zimmermann-positive fractions, the second of which (eluted with 10% aqueous butanol) corresponded to 17-KS sulphates, the third of which (eluted with 15% aqueous (0·1 N NH_4OH) butanol) to 17-KS glucuronides (Table II). Subjected to high voltage paper electrophoresis the 10 % aqueous-butanol fraction revealed the mobility of Rb = 0·86, which is typical for 17-KS sulphates[4]. After cold acid hydrolysis (at pH 0·5) and simultaneous continuous ether extraction (for 48 h) the paper chromatographic evaluation of the above fraction disclosed 3α-hydroxyetiocholan-11,17-dione and higher polar 17-KS (probably 3α,11β-dihydroxy etiocholan-17-one and 3α,11β-dihydroxyandrostan-17-one). The glucuronide fraction (eluted with 15% aqueous

Table II

10 female guinea pigs-urine collection for 6 days. Extraction of conjugates with butanol at pH 11; chromatography of the extract on alumina (according to Crepy[3]).

Eluate	17-Ketosteroids (% of total amount eluted)	Glucuronic Acid
Butanol; 2% aqueous butanol	30–40%	–
6% aqueous butanol	–	–
10% aqueous butanol	40–50%	–
15% aqueous (0·1 N NH_4OH) butanol . . .	3–6%	1·8 mg

[1] F. G. Peron and R. I. Dorfman, J. biol. Chem. *223*, 877 (1956).

[2] T. K. Lakshmanan and S. Lieberman, Arch. Biochem. Biophys. *53*, 258 (1954). – W. Staib and W. Schild, Klin. Wschr. *36*, 166 (1958).

[3] O. Crepy, M. F. Jayle, and F. Meslin, Acta endocrinol. *24*, 233 (1957).

[4] H. Pelzer and W. Staib, Clin. chim. Acta *2*, 407 (1957).

Table I

10 female guinea pigs-urine collection for 6 days. Gradient elution chromatography on alumina after β-glucuronidase hydrolysis and subsequent cold acid hydrolysis (at pH 0·5) with continuous ether extraction (for 48 h).

Total 17-Ketosteroids	Relative distribution of the 17-KS; recorded in % of the total 17-KS-excretion						
15·9 mg	Fraction I 10·3	Fraction II 44·9	Fraction III 5·3	Fraction IV 9·1	Fraction V 10·9	Fraction VI 14·3	Fraction VII 5·2

(0·1 N NH_4OH) butanol) contained only small amounts of 17-KS (3–6% of the total 17-KS), and naphthoresorcine positive material (1·8 mg of glucuronic acid equivalents).

After evaporation of the solvent, fraction one of the above column (eluted with butanol and 2% aqueous butanol) was redissolved in 50 ml of 0·1 m phosphate buffer (pH 7·0) and 3 times extracted with ether. The paper chromatographic analysis of the ether extract showed one pronounced 17-KS zone with the running rate of epiandrosterone, and several smaller 17-KS zones which corresponded to 3α-hydroxyetiocholan-11,17-dione, 3α, 11β-dihydroxyetiocholan-17-one and 3α,11β-dihydroxyandrostan-17-one.

From these findings it may be concluded that the 17-KS in guinea pigs are excreted in the urine partly as sulfuric acid esters; only a small portion appears in the urine as glucuronic acid conjugates. Fraction one of the chromatographic column contained 17-KS as 'free' steroids. It consisted mainly of free epiandrosterone. We are yet unable to determine whether these free steroids were not conjugated primarily or whether a spontaneous hydrolysis of conjugates had taken place in the urine. 17-KS sulphates may easily be hydrolyzed either by spontaneous hydrolysis, or by bacterial influences. In order to cut down on these uncertain disturbing processes we collected the urine under butanol and, until preparation of the extracts began, kept the specimens in a frozen state at −20°C.

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Institute of Physiological Chemistry, Medical Academy of Düsseldorf (Germany), December 22, 1958.

Zusammenfassung

Im Harn von weiblichen Meerschweinchen wurde im Gegensatz zu männlichen Tieren Epiandrosteron als Haupt-17-Ketosteroid nachgewiesen.

Untersuchungen über die 17-Ketosteroidkonjugate im Harn von weiblichen Meerschweinchen ergaben, dass ein grosser Teil (30–40%) als freie Steroide nachweisbar ist. 40–50% liegen als Schwefelsäurekonjugate und 3–6% wahrscheinlich als Glucuronide vor. Bei den konjugierten 17-Ketosteroiden handelt es sich vornehmlich um 11-oxygenierte Steroide.

Corticosteroids and the Radiation Effect in Regenerating Liver of the Rat

The finding that Cortisone inhibited mitosis in regenerating rat liver[1] prompted the suggestion by Professor J. S. Mitchell that part of the inhibitory effect of ionising radiation on mitosis *in vivo* may be due to endogenous corticosteroids.

To test this possibility liver regeneration rates in adrenalectomized rats were compared with normal rats after whole body irradiation.

Method. Male Wistar rats of 180 g were adrenalectomized under ether anesthesia 24 h prior to irradiation. Glucose saline and food given *ad libitum*. Whole body irradiation was given by means of a 220 kV Maximar 15 m.a. tube filtered by 1 mm Al + 1/2 mm Cu at F. S. D. of 1 m. 200 r caused considerable reduction of mitosis rate in normal rat regenerating liver, higher doses adversely affected the adrenalectomized rats. Normal controls shielded by small lead squares over the pituitary and adrenals were irradiated under light Nembutal anesthesia to prevent stress of restraint. Some adrenalectomized rats were shielded in this way to provide strict comparison. The size of lead shields was kept to a minimum though this resulted in imperfect shielding in some rats.

Partial hepatectomy as described by Higgins and Anderson[2] was performed under very light ether anesthesia 1–3 h after irradiation and rats were sacrificed after 30 h by decapitation. Slices of liver from the larger remaining lobes were fixed in Susa, 7 μ paraffin sections cut and stained with haematoxylin and eosin. Phases of mitosis were recorded and counts made of 200 fields which averaged 4000 cells from each liver section. Results are expressed as mitoses per 1000 cells (mitotic index) which is the basis for comparison in these experiments.

Results. Normal Wistar rats show mitotic rates of 40/1000 cells in regenerating liver (Cater, Holmes, and Mee[3]). Unpublished work on this strain of adrenalectomized rats show rates of 60 to 100 mitoses per 1000 cells after partial hepatectomy.

So far 60 adrenalectomized and 60 normal rats have been used but owing to the particularly arduous conditions only 24 adrenalectomized rats have survived. These were in fair condition and showed a mean mitotic rate of 61/1000 cells (± 19 S.D.).

Of the normal rats 54 survived and owing to the difficulties of shielding can be divided into two groups:

(1)—36 rats showing radiation inhibition had low mitosis rates averaging 14/1000 cells (± 4 S.D.).

(2)—18 rats showing high mitosis rates comparable with and having similar histological appearances to adrenalectomized rats. Much of the early shielding has been inadequate and either pituitary or adrenals or both were affected by radiation causing reduced corticosteroid output.

Histological Appearances. Irradiated normal rats show a sharp crenated outline to a pyknotic nucleus and vacuolation of cytoplasm. These changes, with the paucity of mitoses, are similar to those noted in earlier work on unirradiated cortisone treated rats.

Irradiated adrenalectomized rat liver shows degenerating, fragmented, and aberrant nuclear forms with frequent bridges between daughter cells in telophase. Earlier phases show very large 'blown out' forms with occasional shrunk highly pyknotic metaphases. The co-existence of radiation damage and disintegrating cells which still show many mitosing forms, is most striking.

Discussion. 200 r causes considerable reduction of mitoses in normal rat regenerating liver and moderate to severe nuclear damage in both normal and adrenalectomized rats. In spite of this damage a high mitotic rate is seen in liver from adrenalectomized rats, mitoses often proceeding in nuclei obviously breaking down.

The fact that mitosis rate can be separated from nuclear degeneration indicates that the usually accepted radiation effect comprises at least two factors.

(1)—A hormonal inhibition mediated by corticosteroids as part of the general stress response.

(2)—Breakdown of nuclei due to a block in nucleic acid synthesis with accumulation of deoxyribonucleotides as

[1] J. T. Hemingway and D. B. Cater, Nature *181*, 1065 (1958).

[2] G. M. Higgins and R. M. Anderson, Arch. Path. *12*, 186 (1931).

[3] D. B. Cater, B. E. Holmes, and L. K. Mee, Acta radiol. *46*, 655 (1956).

shown by Ord and Stocken[4]. One of the largest accumulations is of deoxycytidine which has been shown by Parizek *et al.*[5] to be excreted in urine in direct proportion to ionizing radiation.

The hormonal inhibition of mitosis by corticosteroids as a result of radiation suggests that cortisone is an adjuvant to radiation in achieving this end and exogenous cortisone used in treating the radiation syndrome should not detract from the therapeutic radiation effect.

Animals with intact adrenals tolerate radiation better as shown by many workers, but this study demonstrates considerably less nuclear degeneration in normal rats than in those adrenalectomised.

My thanks are due to Professor J. S. Mitchell for advice and encouragement, to Dr. K. A. Munday, University of Southampton for facilities and kindness during part of this work and to Miss N. Burr for histological assistance.

J. T. Hemingway

Department of Radiotherapeutics, University of Cambridge, December 1, 1958.

Résumé

La vitesse de régénération du foie chez le rat surrénalectomisé, irradié, est comparée à celle du rat normal radioprotégé, afin d'établir si la sécrétion des corticostéroides est en partie responsable de l'inhibition de la mitose provoquée par la radiation.

Dans le présent travail, il est démontré que le rat surrénalectomisé possède une vitesse de mitose quatre fois supérieure à celle du rat normal, et ceci malgré d'importants dégâts causés au foie par la radiation.

[4] M. G. Ord and L. A. Stocken, Biochem. biophys. Acta *29*, 201 (1958).

[5] J. Parizek, M. Arient, Z. Dienstbier, and J. Skoda, Nature *182*, 721 (1958).

A Contribution on the Mode of Action of D 860

From many reports it is known that hypoglycaemic sulphonylurea compounds are ineffective in depancreatised dogs, rabbits, rats, toads and man, in rabbits and rats with severe alloxan diabetes, and in some severe forms of human diabetes. These facts support the hypothesis which explains the mechanism of the action of hypoglycaemic sulphonylurea compounds in terms of stimulating the beta cells of the islets of Langerhans to increased insulin secretion[1], or by enhancing the action of endogenous insulin[2,3]. Diabetic animals, however, do differ from normal not only in the absence of beta-cells but also in a high glucose concentration in the extracellular fluid. In the present paper we are therefore concerned with the problem of the effect of D 860 on intact rats with hyperglycaemia induced by the exogenous administration of glucose.

Method. Wistar rats were used, weighing 200–300 g and fed on a standard diet, containing 25% protein, 20% fat and 55% carbohydrate (Larsen's diet). Hyperglycaemia was induced by subcutaneous administration of glucose, 180 mg/100 g of body weight, spaced over 20 min intervals, in three fractional doses. In order to avoid any uncontrollable loss of glucose in the urine, the ureters were ligated bilaterally under ether anaesthesia 1 h before the experiment was begun. 2 h after the last dose of glucose, physiological saline was given intravenously to the first group (5 animals); to the second group (5 animals) 0·2 u. insulin (Novo)/kg of body weight was given; to the third group (6 animals) we administered 150 mg/kg D 860 (Artosin, Boehringer) as sodium salt intravenously. To the fourth group (6 animals), insulin 0·2 u. plus D 860 150 mg/kg of body weight was given intravenously. In two groups of intact rats (7 animals) with a normal blood sugar level, we tested the hypoglycaemic action of insulin and D 860. Blood sugar was estimated by a modification of the Somogyi-Nelson method (Frank and Kirberger, 1950).

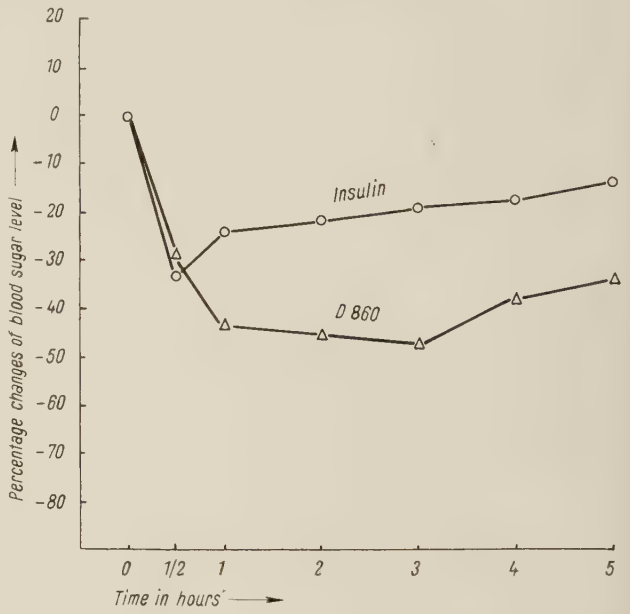

Fig. 1.—Percentage changes in blood sugar levels in rats with a normal initial value (104·7 ± 5·26* and 103·4 ± 4·86 mg% after a single intravenous injection of 0·2 u. insulin, and an intravenous injection of 150 mg D 860/per kg body weight). * S.E. of mean.

Results. Changes of the blood sugar level after a single dose of 0·2 u. insulin/kg body weight or 150 mg D 860 are shown in Figure 1. The maximum decrease in the blood sugar level after insulin occurs after half an hour, and subsequently it returns slowly to the initial level. After the administration of D 860 the blood sugar level decreases by 45%; the fall in blood sugar still being marked and statistically significant ($P < 0{\cdot}001$) 5 h after injection. Figure 2 shows the percentage changes in blood sugar levels after a glucose load. In rats with a normal blood sugar, D 860 caused a drop in blood sugar of 45%. In animals with an initial hyperglycaemia, the same dose leads to a slowing down of the rate of return to normal values. 5 h after the administration of D 860, the blood sugar level is 108% higher than in the group after injection of physiological saline. A single dose of 0·2 u. insulin/kg body weight in animals with an initial hyperglycaemia leads to a more rapid return of the blood sugar to normal. The changes of the blood sugar level after the administration of insulin and D 860 is practically the same as after insulin alone. The blood sugar value after 5 h in the D 860 group was 357 ± 61·1 mg%; in the control group 164 ± 36·8 mg%; in the insulin group 127 ± 21·6 mg%;

[1] A. Loubatières, Ann. N. Y. Acad. Sci. *71*, 192 (1957).

[2] F. Frawley, S. Segal, M. M. Camus, and J. Foley, Ann. N. Y. Acad. Sci. *71*, 81 (1957).

[3] A. Mirsky, G. Perisuti, and R. Jinks, Proc. Soc. exp. Biol. Med. *91*, 475 (1956).

and in the group given insulin and D 860 134 ± 19·2 mg%. The difference of mean of the D 860 group as compared with other groups is statistically significant ($P = 0{\cdot}02$, $P < 0{\cdot}02$, $P < 0{\cdot}01$).

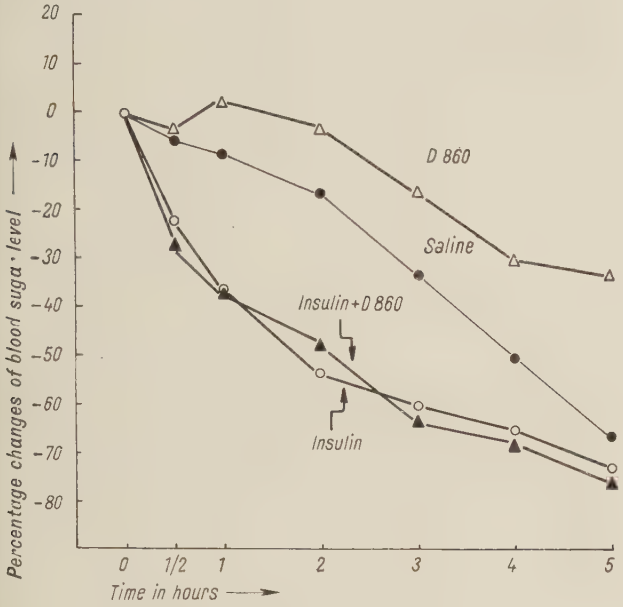

Fig. 2. – Percentage changes in blood sugar levels in rats after a glucose load with initial hyperglycaemia (480 ± 36·2, 402 ± 29·7, 504 ± 34·6, 466 ± 30·1 mg%) and a single intravenous injection of physiological saline, 0·2 u. insulin, 150 mg D 860, 0·2 u. insulin plus 150 mg D 860 (per kg body weight).

Discussion. Hyperglycaemia caused by the administration of glucose leads to degranulation of the beta cells of the pancreas within 15 min[4], which is generally regarded as an indication of increased activity. From our experiments, it is apparent that D 860 did not potentiate the effect of endogenous insulin. It is further obvious that under these experimental conditions no potentiation of the effect of exogenous insulin occurs. The 'paradoxical' effect of D 860 is marked in a single dose which cannot be regarded as toxic. The mechanism of this effect has so far not been eluciated and will be investigated in the future.

It appears, however, that the changes in carbohydrate metabolism after the administration of D 860 and related compounds depend to a great extent on the actual metabolic state of the organism and on the functional activity of peripheral tissues and the liver. From our experiments, it also appears that the ineffectiveness of oral antidiabetics in diabetic animals may be due to hyperglycaemia *per se*. Under these circumstances, we found the effect of insulin and D 860 distinctly different.

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Institute of Human Nutrition, Physiological Department, Prague (Czechoslovakia), January 19, 1959.

Zusammenfassung

Es wird auf die Unwirksamkeit von D 860 bei Ratten hingewiesen, bei denen 2 h vor Verabreichung von D 860 Hyperglykaemie durch parenterale Verfütterung von Glukose hervorgerufen wurde. Die Hypothese wird ausgesprochen, dass Hyperglykaemie einer der Faktoren sein könnte, die die Unwirksamkeit des Sulphonylharnstoffes bewirken.

[4] C. A. Peterson, Proc. Soc. exp. Biol. Med. *70*, 352 (1949).

Hormonale Beeinflussung des Stoffwechsels bei *Carcinus maenas*

Verschiedene Autoren stellten fest, dass bei Krebsen die Grösse des Sauerstoffverbrauches in den einzelnen Phasen des Häutungszyklus verschieden ist. Ergebnisse an einigen Krebsarten lassen darauf schliessen, dass in den neurosekretorisch tätigen Zellen des X-Organes der Augenstiele ein häutungsverhinderndes Hormon gebildet und in den Sinusdrüsen gespeichert wird. Nach Augenstielexstirpation zeigen sich vorzeitige Häutungen; in einigen Fällen wurde zugleich ein Anstieg des Sauerstoffverbrauches beobachtet. Die bisher gefundenen Resultate waren jedoch zum Teil recht unterschiedlich (Darch[1], Scudamore[2], Bliss[3,4], Scheer und Scheer[5]).

Die erwähnten Untersuchungen lassen nicht eindeutig erkennen, ob die Erhöhung des Sauerstoffverbrauches unmittelbar, das heisst durch die Augenstielexstirpation, oder durch einen Anstieg der Bewegungsaktivität während des Häutungsvorganges bedingt ist. Darum wurden an *Carcinus maenas*, an denen meines Wissens noch keine derartigen Untersuchungen durchgeführt wurden, zur Klärung der hormonalen Verhältnisse weitere Experimente gemacht.

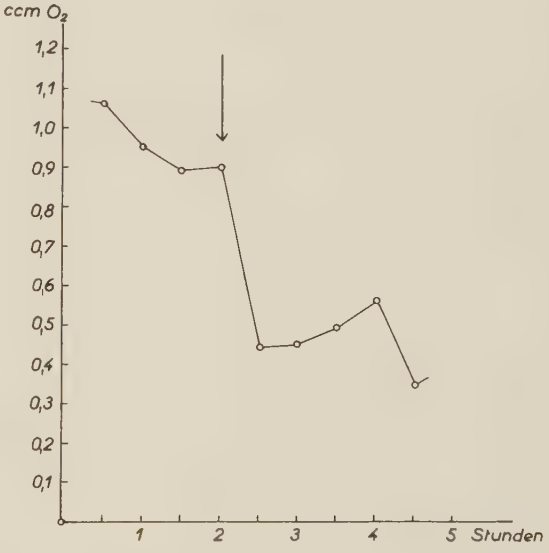

Abb. 1 Der Einfluss von Augenstielextrakten auf die Sauerstoffaufnahme von *Carcinus maenas* (Injektion ↓). Jeder Punkt der Kurve entspricht einer Messung.

Der Sauerstoffverbrauch der Versuchs- und Kontrolltiere wurde halbstündig nach der Methode von Winkler bestimmt. Die Tiere wurden bei einer Temperatur von 12°C gehalten. Nach Feststellung der normalen Durchschnittswerte durch mehrere Messungen wurden die Versuchstiere mit Extrakten aus Augenstielen und die Kontrolltiere mit Seewasser injiziert. Ein Versuchsbeispiel bringt die folgende graphische Darstellung (Abb. 1). Wie hieraus ersichtlich ist, senkte die Injektion von Augenstielextrakten den Sauerstoffbedarf. Die zu gleicher Zeit mit Seewasser injizierten Kontrolltiere hingegen ver-

[1] P. Darch, Biol. France Belg. *78*, 40 (1944).
[2] H. H. Scudamore, Physiol. Zool. *20*, 187 (1947).
[3] D. E. Bliss, Anat. Rec. *111*, 502 (1951).
[4] D. E. Bliss, Biol. Bull. *104*, 275 (1953).
[5] B. T. Scheer und M. A. R. Scheer, Pubbl. Staz. Zool. Napoli *25*, 419 (1954).

brauchten Sauerstoff in normaler Höhe. Eine Verminderung der Bewegungsaktivität der Versuchstiere wurde nicht beobachtet.

Ferner wurde der Sauerstoffverbrauch vor und nach der Augenstielexstirpation gemessen. Dabei zeigte es sich, dass der Bedarf an Sauerstoff bei augenstiellosen Tieren erhöht war, obwohl die normale Bewegungsaktivität nach der Exstirpation wesentlich herabgesetzt war. Ein Versuchsbeispiel zeigt die graphische Darstellung (Abb. 2). Möglicherweise ist der Faktor, der die Häutung verhindert, und derjenige, der den Sauerstoffverbrauch herabsetzt, identisch.

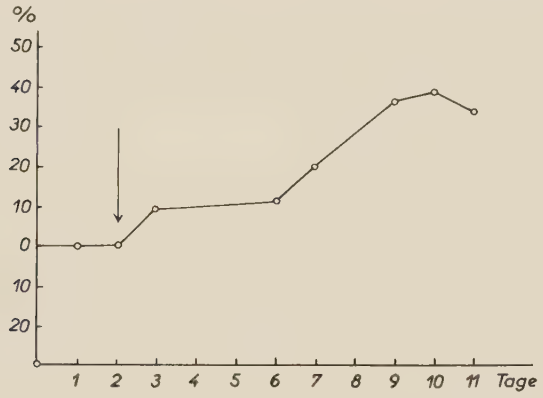

Abb. 2. Der Sauerstoffverbrauch von *Carcinus maenas* nach Augenstiel-Exstirpation (↑) in Prozent der unbehandelten Kontrolle. Jeder Punkt der Kurve entspricht einer Messung.

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Zoologisches Institut der Universität Saarbrücken und Ozeanographisches Institut Monaco, 16. Dezember 1958.

Résumé

L'injection d'extraits de pédoncules oculaires diminue le besoin d'oxygène chez *Carcinus maenas*. D'autre part, la quantité d'oxygène consommé est plus élevée après l'ablation des pédoncules oculaires. Il est probable que le facteur qui inhibe la mue soit aussi responsable de la diminution du besoin d'oxygène.

Atmungsphysiologische Untersuchungen an *Mytilus edulis galloprovincialis*

Aus früheren Arbeiten (Schlieper[1]) geht hervor, dass die Atmung von *Mytilus* durch verschiedene Faktoren beeinflusst werden kann. So kann zum Beispiel die Gonadenentwicklung den Bedarf an Sauerstoff steigern. Brackwassermuscheln zeigen einen wesentlich höheren Sauerstoffverbrauch als Nordseemuscheln (Schlieper). Die Art der Stoffwechsel- und Atmungsregulation der Muschel ist bisher nicht genau geklärt. Sie könnte auf nervösem oder hormonalem Wege erfolgen.

Der Sauerstoffverbrauch der Muscheln wurde nach der Methode von Winkler halbstündig in geschlossenen 1-Liter-Gefässen oder – in einer anderen Versuchsreihe – in einer Durchströmungsapparatur bestimmt. Nach Bestimmung des normalen Sauerstoffverbrauches während eines halben Tages erhielten die Versuchstiere Injektionen aus verschiedenen Organextrakten, die Kontrolltiere gleichzeitig Injektionen mit Seewasser in den Fuss. Sowohl die Versuchs- als auch die Kontrolltiere wurden durch Knebel zwischen den Schalen oder durch Zerschneiden der Schliessmuskeln zur gleichmässigen Atmung gezwungen. Beide Methoden waren in ihrer Wirkung gleichartig.

Nach Vorversuchen mit Injektionen von Adrenalin und Acetylcholin, die keine Wirkung erkennen liessen, wurden die verschiedensten Organextrakte ausgetestet, so zum Beispiel Cerebral- und Visceralganglien, Gonaden, Mitteldarmdrüsen, Pericardialdrüsen, Kiemen und Mantelrand. In keinem Falle konnte eine statistisch gesicherte Erhöhung oder Senkung des Sauerstoffverbrauches nach den Injektionen festgestellt werden. Eine hormonale Beeinflussung des Stoffwechsels durch die getesteten Organe scheint demnach nicht vorzuliegen.

Um eine eventuelle nervöse Regulation der Atmung nachzuweisen, wurden nach Messung des normalen Sauerstoffbedarfes bei einer Gruppe die Visceralganglien, bei einer anderen Gruppe die Cerebralganglien exstirpiert. Mit den Messungen wurde eine Stunde nach den Operationen begonnen. Dabei zeigte sich eine wesentliche Senkung des Sauerstoffverbrauches bei Tieren ohne Visceralganglien. Die Muscheln ohne Cerebralganglien atmeten hingegen fast unverändert weiter. Ein Zahlenbeispiel bringt die folgende Tabelle.

Der Sauerstoffverbrauch von *Mytilus edulis* pro 1 g Lebendgewicht und 1 h, bei 12°C.
(Durchschnittswerte aus je 8 Messungen)

Normal mm^3 O_2	Nach Exstirpation der	
	Visceralganglien mm^3 O_2	Cerebralganglien mm^3 O_2
12,5 ± 1,42	7,64 ± 0,97	11,96 ± 1,34

Die Versuche legen die Vermutung nahe, dass das Visceralganglion bei der Regulation der Atmung beteiligt ist und dieselbe also nervös gesteuert wird.

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Résumé

Chez *Mytilus*, on remarque une baisse sensible de la consommation d'oxygène après l'ablation des ganglions viscéraux, ce qui prouve que ceux-ci jouent un rôle considérable dans la régulation respiratoire.

Importance des modifications de la glycémie dans la régulation de la vitesse d'absorption de solutions de glucose de différentes concentrations chez l'animal intact

Si l'animal intact absorbe le glucose avec une vitesse indépendante de la concentration des solutions[1–3] c'est,

[1] C. Schlieper, Kieler Meeresforsch. *11*, 22 (1955).

[1] D. Cordier, A. Maurice et J. F. Worbe, J. Physiol. fr. *49*, 104 (1957).

[2] P. M. Fullerton et D. S. Parsons, Quarterly J. exp. Physiol. *41*, 410, 531 (1956).

[3] P. C. Reynel et G. H. Spray, J. Physiol. *131*, 452 (1956).

C mg/cm³	N	R	$M(x, y)$	$Po \pm s$	Oo
20	26	−0,76	2,35/146,5	−244 ± 40	227
55	17	−0,93	2,61/223	−469 ± 50	508
150	17	−0,84	2,54/472	−690 ±110	845

dit-on, que la vidange gastrique est inhibée par le glucose présent dans l'estomac, d'autant plus longuement que la concentration est élevée: celle-ci peut varier dans de larges limites de 50 à 250 mg/cm³ selon sans que la vitesse change[3].

Des travaux antérieurs nous ayant conduit à attribuer un rôle régulateur essentiel, et tout à fait indépendant de la vidange gastrique[4] aux modifications de la glycémie[5,6] nous nous sommes demandé comment se comporterait l'animal intact si l'on maintenait sa glycémie constante pendant qu'il absorbe des solutions de differentes concentrations, supprimant ainsi le deuxième mécanisme régulateur, celui de la glycémie, mais non le premier, celui de la vidange gastrique dont nous nous demandons qu'elle peut être l'importance.

Sur le cobaye, avec les techniques décrites[6–8], nous avons comparé trois solutions contenant 20, 55 et 150 mg/cm³, non point à une glycémie unique, mais en faisant un test d'ensemble sur les glycémies comprises entre 100 et 1000 mg %. Utilisant pour cela la corrélation négative qui, nous l'avons montré[7], existe entre les vitesses d'absorption et les glycémies, nous avons calculé, par la méthode des moindres carrés, puis comparé entre elles, les trois droites de régression représentant la fonction: vitesse d'absorption/log. des glycémies.

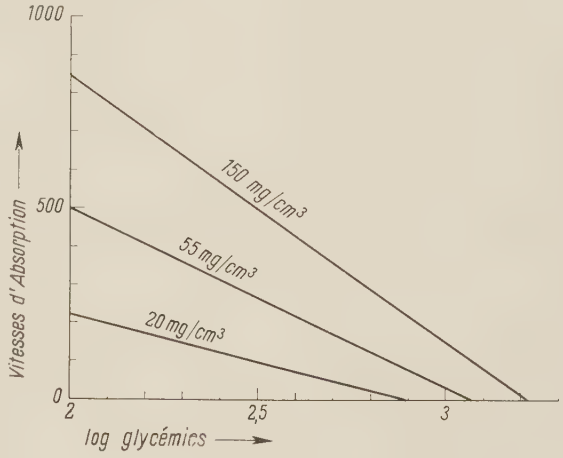
Pour chaque solution, nous avons donc mesuré le glucose absorbé, en 1 h, à des glycémies diverses obtenues par des injections isolées ou simultanées de glucose et d'insuline[7,8]; puis nous avons calculé les éléments du tableau, c'est à dire, avec les concentrations C et les effectifs N, les statistiques des droites de régression y en x: $M(x,y)$ coordonnées des centres de gravité, $Po \pm s$ pentes et écarts types, Oo ordonnées à l'origine.

Les droites ont été tracées dans la Figure (abscisses: log. des glycémies en mg %; ordonnées: vitesses en mg/h).

Les résultats peuvent être résumés ainsi:

1° les trois coefficients de corrélation sont significatifs au seuil de probabilité $1^0/_{00}$ [7].

2° Les droites diffèrent à la fois par leur position dans le plan de Figure et par leur pente. Elles coupent l'axe des x en des régions voisines, mais les pentes croissant en valeur absolue avec les concentrations, elles s'écartent l'une de l'autre dans la région des faibles glycémies. Expérimentalement, cela signifie que les différences de vitesse sont d'autant moins marquées que les glycémies sont plus élevées, ce qui est le cas dans une épreuve d'absorption ordinaire. A ce facteur régulateur s'ajoute le fait que l'hyperglycémie est d'autant plus forte que la solution ingérée est plus concentrée. Maintenant si l'on se place à une glycémie normale (100 mg %) les vitesses, qui, dans la Figure, sont représentées par les ordonnées à l'origine, sont de 227, 508, 845 mg/h. Elles augmentent donc de 4 fois, et pour les deux dernières solutions qui, dans les expériences citées en [3] étaient absorbées à la même vitesse, nous trouvons une différence de 66%.



Il est possible qu'en augmentant les concentrations, les vitesses atteignent une limite. Mais pour les solutions utilisées, notre schéma expérimental montre que la seule présence de glucose dans l'estomac n'inhibe pas la vidange gastrique au point de rendre la vitesse d'absorption indépendante de la concentration. Ce sont les modifications de la glycémie qui jouent un rôle déterminant: ce sont elles qui ralentissent l'absorption et empêchent l'estomac de délivrer de nouvelles quantités de glucose. L'inhibition de la vidange gastrique apparaît ainsi comme la conséquence du défaut d'absorption et non comme sa cause.

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Institut de Biologie, Service de Physiologie, Paris, le 22 décembre 1958.

Summary

In intact guinea-pigs fed with glucose solutions of different concentrations, the main limiting factor of absorption is not the gastric emptying but the hyperglycaemia.

Response of Adrenal and Preputial Glands of Rats to Administration of 5-Hydroxytryptamine

It is well known that 5-hydroxytryptamine (5-HT) induces enlargement of the adrenal glands[1]. Hypertrophy of the adrenals and increased urinary output of 17-hydroxycorticoids after administration of reserpine, a 5-HT liberator, have been reported[2]. Moreover, it has been suggested that adrenal glucocorticoids may exert a functional control of the tissue level of 5-HT[3].

The relationship between 5-HT and the endocrine system has not been extensively studied, however. For

[4] M. Lourau, C. R. Acad. Sci., Paris, *238*, 936 (1954).
[5] M. Lourau et O. Lartigue, C. R. Acad. Sci., Paris, *232*, 1697 (1951).
[6] M. Lourau, C. R. Acad. Sci., Paris, *238*, 842 (1954).
[7] M. Lourau et O. Lartigue, Exper. *7*, 428 (1951).
[8] M. Lourau, C. R. Acad. Sci., Paris, *236*, 1376 (1953).

[1] C. Casella and G. Rapuzzi, Boll. Soc. Ital. Biol. Sper. *33*, 722 (1957).
[2] R. H. Egdahl, J. B. Richards, and D. M. Hume, Science *123*, 418 (1956). — R. Gaunt, A. A. Renzi, N. Antonchak, G. J. Miller, and M. Gilman, Ann. N. Y. Acad. Sci. *59*, 22 (1954).
[3] R. Hicks and G. B. West, Nature *181*, 1342 (1958).

Weight and Ascorbic Acid Content of Adrenal and Preputial Glands of Intact, Ovariectomized, Adrenalectomized and Ovariectomized-Adrenalectomized Female Rats, After a Single Injection of 5-HT.

	Body weight g	Adrenal gland weight mg	Adrenal ascorbic acid µg/100 mg tiss.	Preputial gland weight mg	Preputial ascorbic acid µg/100 mg tiss.
Intact					
Controls	162	50·3 ± 9·5	280·2 ± 43·9	52·1 ± 13·6	69·9 ± 15·4
Injected with 5HT	161	54·3 ± 7·4	214·8 ± 33·4	65·6 ± 18·8	61 ± 24
% variation		+ 7·9	− 23·4	+ 25·9	− 12·8
		$p < 0.5$	$p < 0.01$	$p < 0.1$	$p < 0.5$
Ovariectomized					
Controls	153	50·2 ± 7·2	398·5 ± 39·2	47·1 ± 7·1	104·4 ± 20·4
Injected with 5HT	154	48·7 ± 5	307·1 ± 66·6	51·7 ± 16·5	84·9 ± 20·4
% variation		− 3	− 23	+ 9·7	− 18·7
			$p < 0.001$	$p < 0.5$	$p < 0.1$
Adrenalectomized					
Controls	154			62·7 ± 9·8	92·6 ± 10·7
Injected with 5HT	153			79·5 ± 23·5	75·7 ± 14
% variation				+ 26·7	− 18·3
				$p < 0.05$	$p < 0.01$
Ovariectomized-Adrenalectomized					
Controls	150			55·3 ± 18·6	114·3 ± 30·6
Injected with 5HT	154			62 ± 18·7	89·6 ± 22·5
% variation				+ 12·1	− 21·7
				$p < 0.5$	$p < 0.05$

this purpose the effects of a single injection of 5-HT on the adrenal and preputial glands were examined, the latter being considered a target organ of corticotrophin action[4]. In order to evaluate this response, the endocrine sources of steroid hormones (viz. ovaries and adrenals) were removed.

Material and methods. Eighty albino female Wistar rats with a body weight of 150–160 g were employed. They were kept on Rockland diet and fed *ad libitum*. Four series were treated: intact, ovariectomized, adrenalectomized, and ovariectomized-adrenalectomized. The surgical operations were performed under ether anaesthesia four days before the beginning of the experiments. Adrenalectomized rats were maintained on drinking fluid containing 1% sodium chloride. Each series consisted of ten rats treated with 5-HT injected subcutaneously and ten controls. The dose was 10 mg/kg dissolved in saline[5]. The controls received saline only. All rats were sacrificed by decapitation 2 h after the injection. The adrenal and preputial glands were weighed and the percentage values of their ascorbic acid content determined[6].

Results. The data are summarized in the Table. The weight of the adrenals was unchanged after the 5-HT treatment. The adrenal ascorbic acid content was remarkably decreased, both in intact and ovariectomized rats (23·4% and 23·0% respectively). The increase in preputial gland weight was 25·7% in the series of intact rats, being almost unchanged in the adrenalectomized (26·7%). The increase was less, however, in ovariectomized and ovariectomized-adrenalectomized rats (9·7% and 12·1% respectively). The preputial ascorbic acid content was decreased in all series, most remarkably in those operated on.

Discussion. The adrenal ascorbic acid depletion observed in this experiment is in accordance with the results obtained by Bertelli *et al.*[7], and seems to confirm previous reports concerning the effects of 5-HT treatment on the pituitary-adrenal axis, mediated by an ACTH-releasing effect[8]. The mediation by ACTH was also suggested by the observation that 5-HT induces no depletion of adrenal ascorbic acid in hypophysectomized rats[8] and by lacking eosinopenic response in adrenalectomized ones[9].

In the present experiment, a parallel depletion, although less marked, was found in the ascorbic acid content of the preputial glands after administration of 5-HT. This is in accordance with the results obtained by Hess *et al.*[10] concerning the depletion of preputial ascorbic acid induced by severe stress in intact rats, and by high doses of ACTH in hypophysectomized rats.

Moreover, an increase in perputial weight was observed in all series after injection of 5-HT. This increase was moderate in the ovariectomized animals (9·7%), but remarkable in the adrenalectomized (26·7%). Thus, through an uninhibited ACTH liberation, simple adrenalectomy may induce an increase in weight of the preputial glands (20·3% as compared with the controls of the intact series). The still higher increase in preputial weight of adrenalectomized rats injected with 5-HT (52·5%) also indicates an ACTH-releasing effect of this substance.

The ovariectomy, on the contrary, seems to inhibit the increase in preputial weight induced by 5-HT treatmens. We notify, however, that in another group of rats (not

[4] B. Jacot and H. Selye, Endocrinology *50*, 254 (1952).

[5] 5-Hydroxytryptamine creatinine sulphate was kindly supplied by Farmitalia S.p.A. Milan, Italy.

[6] The ascorbic acid was determined according the E. Martini and A. Bonsignore's method, Boll. Soc. Ital. Biol. Sper. *19*, 338 (1934), with the modifications reported by R. Margaria, *Principi di chimica e fisico-chimica fisiologica* (CEA, Milano 1952).

[7] A. Bertelli, G. Cantone, and L. Martini, Atti Soc. Lomb. Sc. Med. Biol. *9*, 10 (1954). – G. Georges, C. R. Soc. Biol. *151*, 692 (1957). – P. G. Smelik and D. DeWied, Exper. *14*, 17 (1958). – E. Costa and G. Zetler, Proc. Soc. exp. Biol. Med. *98*, 249 (1958).

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[9] F. A. Steiner and Chr. Hedinger, Exper. *12*, 109 (1956). – F. A. Steiner, R. E. Siebenmann, C. Sandri, and Chr. Hedinger, Exper. *13*, 500 (1957).

[10] M. Hess, O. Hall, C. E. Hall, and J. C. Finerty, Proc. Soc. exp. Biol. Med. *79*, 290 (1952).

reported in the Table), the simple laparotomy induced a decrease of 14·3% in the weight of preputial glands.

L. Fiore-Donati, L. Pollice, and
L. Chieco-Bianchi

Institute of Pathological Anatomy, University of Bari (Italy), January 6, 1959.

Zusammenfassung

An weiblichen intakten, adrenal- oder ovariektomierten und sowohl adrenal-, als auch ovariektomierten Ratten wurden 2 h nach Injektion von 5-Hydroxytryptamin die Gewichtsveränderungen und der Ascorbinsäuregehalt der Nebennieren und der Präputialdrüsen untersucht. Eine Abnahme des Ascorbinsäuregehaltes der Nebennieren und der Präputialdrüsen wurde festgestellt und eine Gewichtszunahme der Präputialdrüsen selber beobachtet. Die Ergebnisse stimmen mit den bekannten Befunden über eine ACTH-freisetzende Tätigkeit des 5-Hydroxytryptamins überein.

Identification of a Third Subdivision of the Dorsal Spino-Cerebellar Tract

Ascending tracts in the dorso-lateral funiculus (Flechsig's fasciculus) have been analyzed by recording the mass discharge from the dissected fasciculus and by intracellular recording from single fibres. Four types of ascending pathways have been differentiated[1].

A. Neurones monosynaptically activated by impulses in muscle spindle afferents (Ia and II).

B. Neurones monosynaptically activated by impulses in Golgi tendon organ afferents.

C. Neurones activated by impulses in group II and III muscle afferents, and also by impulses in skin and joint afferents. These excitatory actions are drawn from an extremely wide ipsilateral receptive field. On adequate stimulation, a discharge can be evoked by muscle stretch and also from large areas of skin (usually by pressure and pinching, but sometimes also by bending of hairs).

D. Neurones activated exclusively by ipsilateral low threshold cutaneous afferents from a very restricted receptive field, the discharge being adequately elicited by bending of hairs.

There was reason to assume that type A and B constitute the dorsal spino-cerebellar tract, because, on stimulation of group I muscle afferents, a potential can be recorded from the anterior cerebellar vermis, which corresponds to the discharge in these neurones[1]. Stimulation of group II and III muscle afferents, on the other hand, did not evoke a potential change in the cerebellar cortex; hence it could not be assumed that the axons of type C terminated in the cerebellar cortex.

In the present experiments, a more direct approach has been used for identification of axons belonging to the dorsal spino-cerebellar tract by investigating whether they can be activated antidromically from the cerebellar cortex.

Of 67 neurones belonging to type A and B, 50 could be activated antidromically from the anterior vermis. Those 17 axons which could not be activated antidromically may have terminated in the most rostral part of the vermis, not exposed for stimulation. None of 47 axons tested could be activated by stimulation of the dorso-lateral funiculus in the proximal L V segment, as would be expected since Clarke's column does not extend caudally below the L IV segment.

63 neurones belonging to type C were identified; 36 of them could be activated antidromically from the anterior cerebellar vermis. Stimulation of the dorso-lateral funiculus in upper L V failed to activate the axon of any type C neurone with connection to cerebellum. Hence it can be concluded that neurones of type C constitute a third subdivision of the dorsal spino-cerebellar tract.

However, it is noteworthy that the proportion of neurones not activated antidromically from the cerebellum is larger with type C than with A and B (43 resp. 25%). There is, indeed, evidence of another ascending system of type C because of 22 neurones, which could not be activated from cerebellum, 11 were activated on stimulation of the dorso-lateral funiculus in L V and consequently have their cell bodies below the caudal end of Clarke's column. No experiments have been made to find the termination of these axons.

None of the units of type D could be activated antidromically from the cerebellum, and with all of these neurones the axons could be stimulated in L V.

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Institute of Physiology, University of Lund (Sweden), January 5, 1959.

Zusammenfassung

Aktionspotentiale einzelner Fasern des Funiculus dorsolateralis des Rückenmarks wurden registriert und dabei nach Axonen gesucht, die antidrom von der Kleinhirnrinde erregt werden konnten. Es gelang die Identifizierung einer dritten Unterabteilung der dorsalen spino-cerebellaren Bahn. Diese Neurone werden sowohl durch Muskelafferente der Gruppen II und III als auch durch Haut- und Gelenkafferente erregt. Zwei andere aufsteigende Bahnen, deren Zellkörper unterhalb des caudalen Endes der Clarkschen Säule liegen und deren Axonen sich in dem Funiculus dorsolateralis befinden, erreichen die Kleinhirnrinde nicht.

The Relationship Between the Flexion Reflex and Certain Ascending Spinal Pathways

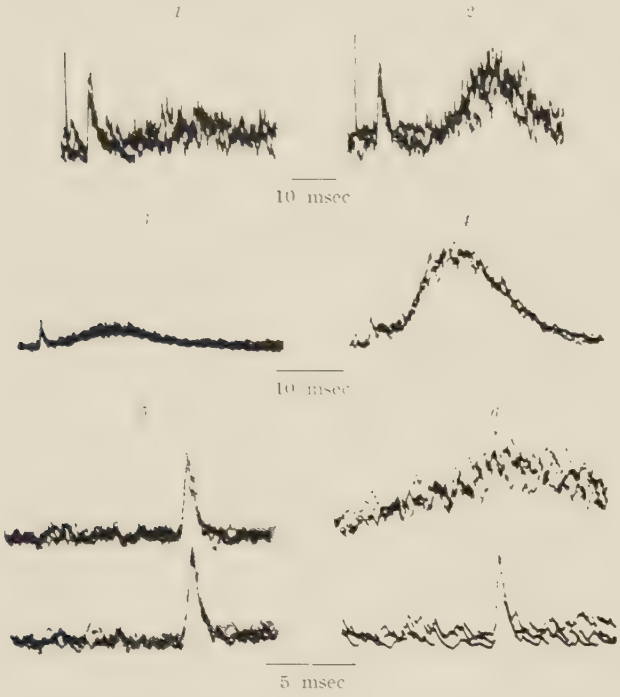
In spinal cats, impulses in group II and III muscle afferents, in skin and in high threshold joint afferents give rise to the flexion reflex with excitation to flexor and inhibition to extensor motoneurones[1]. In decerebrate cats, these actions are markedly depressed or even absent because the interneurones mediating them are tonically inhibited from suprasegmental centres[2].

[1] Y. Laporte, A. Lundberg, and O. Oscarsson, Acta physiol. scand. *36*, 175, 187 (1956).—Y. Laporte and A. Lundberg, Acta physiol. scand. *36*, 203 (1956).—A. Lundberg and O. Oscarsson, Acta physiol. scand. *38*, 53 (1956).—B. Holmqvist, A. Lundberg, and O. Oscarsson, Acta physiol. scand. *38*, 75 (1956).—O. Oscarsson, Arch. ital. Biol. *96*, 199 (1958).

[1] D. P. C. Lloyd, J. Neurophysiol. *6*, 293 (1943).–L. G. Brock, J. C. Eccles, and W. Rall, Proc. Roy. Soc. London (B) *138*, 453 (1951).–R. M. Eccles and A. Lundberg, to be published.

[2] R. M. Eccles and A. Lundberg, Exper. *14*, 197 (1958).

The present investigation was developed also in relationship to the problem of the functional significance of ascending pathways influenced by these afferents. Four such pathways (*A*–*D*) are known at present: *A* and *B* have their axons in the dorsal part of the lateral funicle and the receptive field is ipsilateral[3]. Pathway *A* is a subdivision of the dorsal spino-cerebellar tract, whereas the axons of pathway *B* have their cellbodies below the caudal end of Clarke's column. The termination of the axons of pathway *B* is unknown.



Comparison of discharge in ascending spinal tracts in decerebrate cats before (left column) and after (right column) section of the spinal cord. Records 1 and 2 show the discharge in the dissected Flechsig's fasciculus on stimulation of the ipsilateral hamstring nerve. Records 3 and 4 were obtained from the dissected spinal half (except the dorsal column) on stimulation of the contralateral hamstring nerve. Observe that the early components of the discharge, due to activity in group I activated neurones of the DSCT (1 and 2) and of the VSCT (3 and 4) have not changed after section of the cord.

Records 5 and 6 show the VSCT discharge recorded from the right dissected spinal half on stimulation of the left hamstring nerve. The unconditioned discharges are shown in lower traces; the upper traces were obtained after a conditioning volley in the left gastrocnemius nerve (interval between conditioning and testing volleys 12 msec).

The axons of pathway *C* are located in the ventral quadrant of the cord and the cell bodies in the lower lumbar segments. The receptive field is bilateral, the axons do reach the brain stem but their termination is unknown[4].

The neurones of pathway *A*, *B* and *C* receive excitatory action from group II and III muscle afferents, from skin, and from high threshold joint afferents. The receptive field is extremely wide and includes antagonist muscles and large areas of skin. The neurones usually respond to a single volley in a peripheral nerve with a train of impulses, and, on adequate stimulation, there is a long-lasting after-discharge, such as would be expected with actions mediated through a chain of interneurones.

Pathway *D* is the ventral spino-cerebellar tract (VSCT). Its neurones are monosynaptically excited by Golgi tendon organ afferents, but, in addition, in the majority of VSCT neurones impulses in the afferents giving rise to the flexion reflex evoke, through polysynaptic pathways, inhibition. Also in this case, the receptive field is very wide[5].

The description above refers to spinal cats and, in the present investigation, transmission to these tracts have been compared in decerebrate and spinal cats (Figure). Records 1 and 2 were obtained from the dissected dorsal part of the lateral funicle and show the discharge in pathway *A* + *B* before (record 1) and after (record 2) section of the spinal cord. The large release indicates that transmission to pathway *A* and *B* is tonically inhibited from suprasegmental centres in the non-spinal state. An equally large release is found in the corresponding records 3 and 4, showing the discharge in pathway *C* as recorded from the dissected right spinal half on stimulation of the left hamstring nerve. A release of inhibitory action on to VSCT neurones after cord section is illustrated in records 5 and 6. The VSCT discharge, evoked on stimulation of Ib afferents in the hamstring nerve, was recorded from the dissected spinal half (unconditioned discharge in lower traces of records 5 and 6). The upper traces were obtained after a conditioning volley had been evoked in the gastrocnemius nerve. The large release of inhibitory action after section of the remaining intact half of the cord shows that the interneurones mediating this action are tonically inhibited in the non-spinal state.

Further experiments have revealed that the control of these four ascending pathways is similarly organized as the suprasegmental inhibitory control of the flexion reflex. In both instances, the control is unaffected by stimulation of the anterior lobe of cerebellum leading to complete collapse of decerebrate rigidity or by ablation of cerebellum. It does not decrease after bilateral destruction of the vestibular nuclei but disappears after a lesion at obex. The descending tracts responsible for this control are in all cases located in the dorsal part of the lateral funicles, and from either side a bilateral effect is exerted.

As regards the functional significance of these four pathways, it should be noted that they can neither be classified as exteroceptive nor as proprioceptive. Their connection with primary afferents rather associates them with the flexion reflex. The similarity in supraspinal control of these pathways and of the flexion reflex suggests that they may convey information regarding the state of the flexion reflex, as decided both by the receptor activity and the degree of supraspinal control of the flexion reflex interneurones. Such information may be of general importance in the reflex taxis of the animal, or it may be more specifically utilized by supraspinal centres in regulation of this reflex.

B. Holmqvist, A. Lundberg, and O. Oscarsson

Institute of Physiology, Lund, January 31, 1959.

Zusammenfassung

Vier aufsteigende Rückenmarksbahnen werden bei spinalen Katzen durch somatische Afferenzen, die den Flexorreflex auslösen, beeinflusst. Genau wie beim Flexorreflex ist die Übertragung auf diese Bahnen bei dezerebrierten Katzen stark gehemmt. Die Regulierung der supraspinalen Hemmung der Bahnen und des Flexorreflexes scheint in gleicher Weise organisiert zu sein.

[3] Y. Laporte, A. Lundberg, and O. Oscarsson, Acta physiol. Scand. *36*, 188 (1956).–A. Lundberg and O. Oscarsson, Exper. *15*, 195 (1959).

[4] O. Oscarsson, Arch. ital. Biol. *96*, 199 (1958).

[5] O. Oscarsson, Acta physiol. Scand. *42*, Suppl. 146 (1957).

The Electrophoretic Distribution of Protein-bound Carbohydrates During Attacks of Asthma Bronchiale

In a previous communication[1], the correlation of protein-bound carbohydrates (PBC) and their electrophoretic distribution to bronchial asthma was examined. Two types of distribution were found, half of our cases showing no significant changes at all, the other half being of the 'albuminotropic type', closely correlated to eosinophilia. These results were obtained from patients while no attack was in progress. We were therefore interested in possible changes in the electropherograms of PBCs in patients during an asthmatic attack, in comparison with the findings between the attacks.

12 typical adult cases with severe asthma bronchiale were examined. The routine laboratory findings were normal (excepted occasional eosinophilia). The electrophoresis of the PBCs and their staining was performed according to Köiw[2] with our modification. In 10 cases out of 12, a significant increase of the PBCs in the α-2-globulins was found. The significance was calculated to our own normal values.

The observed increase in the α-2-globulin fraction is of no specificity at all, but has been described (Bernasconi[3]) after the administration of ACTH, and does not depend upon the functional integrity of the suprarenal cortex.

Indeed, cases of bronchial asthma of long duration and with severe and frequent attacks show a relative corticoid insufficiency. In our patients, the mean value of neutral 17-ketosteroids in urine was 7·6 mg/24 h (after application of Prednisone, even lower values were observed!).

In conclusion, we found in adult patients with severe asthma bronchialis, a transitory increase of PBCs in the α-2-globulins during the asthmatic attack. This could be explained as a result of the stress effect, followed by an output of ACTH. The relatively insufficient suprarenal cortex does not respond adequately and the asthmatic attack occurs.

We did not find an increase of the PBCs in the γ-globulin fraction; but it must be noted that the method used only reveals quantitative and not qualitative changes.

Attention must be drawn to the possibility that antibodies, containing PBCs and precipitating the attacks, may move together with the α-2-globulins, thus increasing their action. This is another explanation of our results.

T. Kološ and J. Kellen

Laboratory of the National Spa Štrbské Pleso and Laboratory of the National Spa Nový Smokovec (Czechoslovakia), February 9, 1959.

Zusammenfassung

Das elektrophoretische Glykoproteinogramm zeigt während schwerer Anfälle von Asthma bronchiale bei der Mehrzahl der Patienten eine signifikante Zunahme der Glykoproteine in der α-2-Globulinfraktion, was als Stresseffekt gedeutet wird. Zudem besteht die Möglichkeit, dass den Anfall auslösende Antikörper selber Glykoproteine enthalten und, da sie mit den α-2-Globulinen wandern, für die Zunahme dieser Fraktion verantwortlich sind.

[1] J. Kellen and T. Kološ, Exper. *14*, 375 (1958).

[2] E. Köiw and A. Grönwall, Scand. J. clin. Lab. Invest. *4*, 244 (1952).

[3] C. Bernasconi, Acta endocrinol *25*, 50 (1957).

Der Einfluss verschiedener Psychopharmaka auf den Bluthistamingehalt von Ratten

Über den Histamingehalt des Rattenblutes ist wenig bekannt, doch wird angenommen, dass er, verglichen mit anderen Tierspezies, ziemlich hoch sei. Emmelin[1] fand 0,1–0,33 μg/ml im Plasma, Halpern[2] viel weniger (0,036 μg/ml). Rose und Browne[3] fanden im Gesamtblut jedoch nur 0,035–0,06 μg/ml.

Wir haben mit der kolorimetrischen Mikromethode von Lowry *et al.*[4] das Gesamthistamin im Vollblut und im Plasma von Ratten nach Extraktion mit Trichloressigsäure bestimmt und in Parallelversuchen mit der biologischen Methode von Code[5] am Meerschweinchendarm verglichen. Im *Vollblut* fanden wir bei jüngeren Ratten (150–200 g) 0,185 ± 0,051 μg/ml Histamin, bei älteren Ratten (250–350 g) 0,236 ± 0,022 μg/ml in guter Übereinstimmung beider Methoden. Das *Plasma* der jungen Tiere enthielt davon einen überraschend grossen Anteil von 0,071 ± 0,0046 μg/ml Histamin. Da alle Thrombozyten durch 10 min Zentrifugieren bei 500 g und 10 min bei 1500 g entfernt wurden und die Thrombozyten der Ratten verschwindend wenig Histamin enthalten, kann dieser relativ hohe Anteil nicht durch Thrombozyten verursacht sein. Die quantitativen Verhältnisse sind daher denjenigen des Hundeplasmas (0,04–0,07 μg/ml, Barsoum und Gaddum[6]) ähnlich. Die Leukozyten scheinen ziemlich viel Histamin zu enthalten.

Die Wirkung der verschiedenen Pharmaka wurde an Rattengruppen von je 16–18 Tieren (150–200 g) im Zeitpunkt der stärksten Wirkung untersucht. Das Blut wurde durch Halsschnitt entnommen, in einem Gefäss mit Oxalat aufgefangen und sofort 2mal wie angegeben zentrifugiert. Nach 30 min Extraktion mit 30% Trichloressigsäure wurde nochmals während 20 min mit 1500 g zentrifugiert und der überstehende Plasmaextrakt mit der kolorimetrischen Methode nach Lowry[4] untersucht.

Durch Narkotika wurde der Histamingehalt im Plasma im Toleranzstadium unterschiedlich beeinflusst. Einstündige *Äther*narkose senkte ihn auf 75% (Tabelle), während *Chloroform*narkose wirkungslos blieb.

Typische Tranquilizer wie *Reserpin* oder *Chlorpromazin* verursachen eine deutliche Erhöhung um 14–25%. Da in unseren Versuchen der zeitliche Verlauf nicht untersucht wurde, kann der Unterschied zwischen beiden Pharmaka rein zufällig sein. Auch 1 h nach *Numal* ist Histamin im Plasma leicht erhöht.

Psychotomimetische Stoffe können den Histamingehalt erhöhen oder senken. *Iproniazid* hemmt nicht nur die Monoaminoxydase, sondern auch die Diaminoxydase (Zeller *et al.*[7], Schayer[8]) und damit den Histaminabbau. Dadurch, vielleicht aber auch durch erhöhte Histaminfreisetzung aus dem Gewebe, wird der hohe Blutgehalt nach 24 h (+ 44%) erklärt. Das deutlich erregende *Amphetamin* hat nach 5 h eine viel geringere Steigerung

[1] N. Emmelin, Acta physiol. scand. *11*, Suppl. 34, 1 (1945).

[2] B. N. Halpern, *Histamine*, Ciba Foundation Symposium (Churchill, London 1956), p. 109.

[3] B. Rose und J. S. L. Browne, Amer. J. Physiol. *124*, 412 (1938).

[4] O. H. Lowry, H. T. Graham, F. B. Harris, M. K. Priebat, A. R. Marks und R. U. Bregman, J. pharm. exp. Therap. *112*, 116 (1954).

[5] C. F. Code, J. Physiol. *89*, 257 (1937).

[6] G. S. Barsoum and J. H. Gaddum, J. Physiol. *85*, 1 (1935).

[7] E. A. Zeller, J. Barsky, J. R. Fouts, W. F. Kirchheimer und L. S. van Orden, Exper. *8*, 349 (1952).

[8] R. W. Schayer, J. biol. Chem. *203*, 787 (1953).

Tabelle

Pharmaka	Dosis mg/kg	Wirkungsdauer h	Histamingehalt µg/ml	% des Normalhistamingehaltes	*P*
Normalplasma			0,071 ± 0,0046	100	
Äther		1	0,054 ± 0,0026	75	< 0,001
Chloroform		1	0,068 ± 0,0048	95	0,6
Reserpin	1,0	3	0,081 ± 0,0052	114	0,001
Chlorpromazin	10,0	3	0,088 ± 0,0060	125	< 0,001
Numal	0,1	1	0,078 ± 0,0072	110	0,02
Iproniazid	100,0	24	0,102 ± 0,0044	144	< 0,001
Amphetamin	5,0	3	0,080 ± 0,0031	113	< 0,001
Mescalin	25,0	4	0,066 ± 0,0121	92	0,1–0,2
LSD	2,0	2	0,045 ± 0,0052	63	< 0,001

(+ 13%) zur Folge; eine Hemmwirkung auf die Aminoxydase ist unsicher. Die beiden bekanntesten Phantastika senken den Plasmahistamingehalt: *Mescalin* nur wenig und statistisch nicht sicher, aber *LSD* schon nach 2 h um 40%! Dieser Unterschied ist vor allem, verglichen mit der stark sedativen Wirkung der Antagonisten Reserpin, Chlorpromazin und Numal, interessant, da innerhalb dieser Gruppe eine Parallelität zwischen psychischer Wirkung und Histaminstoffwechsel besteht, was bei der Katecholamin- und Serotoninfreisetzung bekanntlich nicht der Fall ist.

Immerhin muss betont werden, dass die psychopharmakologischen Effekte aller untersuchten Stoffe keineswegs mit dem Plasmagehalt an Histamin vergleichbar sind. Doch wissen wir über die Beeinflussung der Bildung, der Freisetzung, des fermentativen Abbaus, der Ausscheidung des Histamins, wie auch über den zeitlichen Verlauf und den Ort dieser Wirkung noch sehr wenig.

P. G. Waser und M. Itzbicki

Pharmakologisches Institut der Universität Zürich, 9. Februar 1959.

Summary

The concentration of histamine in plasma of rats is changed by injection of psychopharmacons as follows: decreased by narcotics (ether, chloroform) and phantastics (LSD, mescaline), increased by tranquilizers (reserpine, chlorpromazine) a hypnotic (numal) and psychotomimetics (iproniacide, amphetamine).

PRO LABORATORIO

Apparatus for Spectrophotometric Microtitration Application to the Titration of Deuterohemin

In 1955, we built a small apparatus replacing the standard cell holder of a Beckman spectrophotometer Mod. DU, which allows concomitant measurement of the extinction and of the pH. A similiar arrangement has been recently described by Boaz and Forbes[1] for the 'Cary' recording spectrophotometer. This encouraged us to publish the present note.

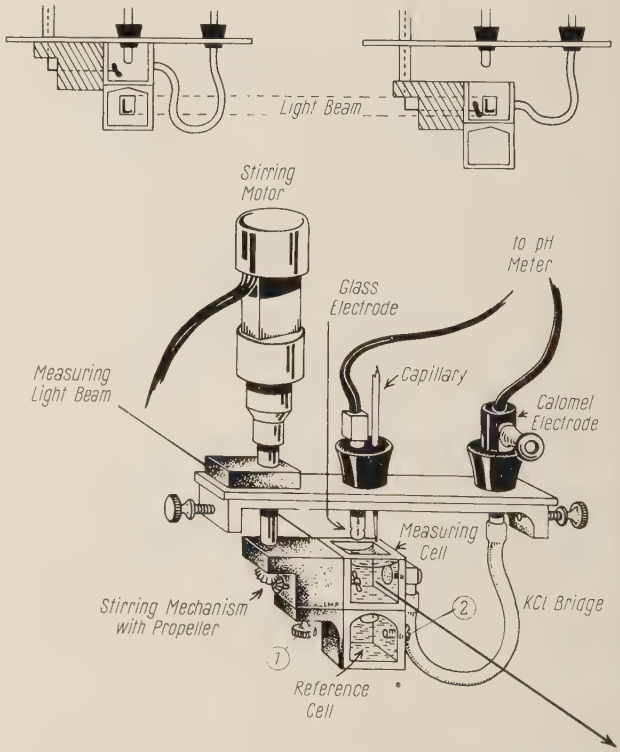


Fig. 1.—*Sketch of the apparatus.* The lock (made of brass) fits into the standard cell-holder compartment of a Beckman DU spectrophotometer. The lock carries: (1) the stirring motor, to which the cells (made of lucite) are attached; (2) a rubber stopper with the glass electrode and capillary from the micro-buret; and (3) a rubber stopper with a calomel electrode connected to KCl-bridge. The electrodes are by Beckman.—On top of the figure, the operation of the apparatus is schematically represented. For further explanations cf. the text.

The requirements of simplicity, light-tightness, and protection of delicate components (glass electrode and tip of microburet) demanded a minimum of moving parts. The sample and reference cells, made of lucite, are placed on top of each other and are movable in a vertical direction (Fig. 1). The glass electrode and the capillary tip of the

[1] H. E. Boaz and J. W. Forbes, Anal. Chem. *30*, 456 (1958).

microburet (an 'Agla' syringe from Borroughs-Wellcome) remain fixed. The calomel electrode is connected to the sample cell by a piece of tygon tubing filled with KCl solution. Liquid connection between this salt bridge and the sample solution is made through small holes in the lucite cell wall plugged with cotton thread. Stirring is accomplished by a tiny propeller of pure nickel, driven by a small electric motor which also serves as a handle for raising and lowering the two cells.

Measurements are made as follows: the apparatus is removed from the spectrophotometer. The reference cell is disconnected [screw (1)] and filled at (2) by means of a syringe. After closing (2) with a threaded teflon plug, the cell is attached again. Gas bubbles collect in the roof of the cell. The KCl bridge is now freed from air bubbles, the parts reassembled, and checked for light leaks (black tape).

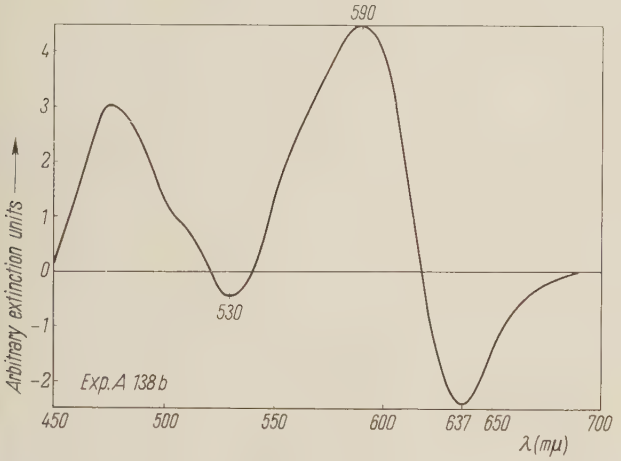


Fig. 2*a*.—The difference between the absorption spectra of deuterohemin at pH 10·3 (borate buffer) and at pH 5·7 (acetate buffer) respectively [Deuterohemin] ≈ 0·02 m*M*. The wavelengths 530 and 590 mμ were chosen for the titration experiment.

Abscissa: wavelength in mμ; ordinate: arbitrary extinction units (difference values).

Readings are made in 2 positions of the cell holder, as shown at the top of Figure 1. In *position A* (at left) an addition of the titrating solution to the sample solution is made, followed by 10–15 sec of stirring. The pH of the sample solution and the extinction of the reference solution are measured ('check' position of the spectrophotometer). In *position B* (at right) the extinction of the sample solution is read, whereupon a new 'cycle' is started.

The assembly was used for titrating deuterohemin (deallyl-protohemin IX). A difference spectrum (Fig. 2*A*) showed the two wavelengths at which the extinctions of deuterohemin at pH 5·7 and at pH 10·3 differ the most, namely 530 mμ and 590 mμ. The spectrophotometric titration was therefore carried out at these 2 wave lengths.

Pure, recrystallized deuterohemin was obtained by heating protohemin with three parts of resorcinol at 200° for 45 min and recrystallizing the crude product from warm acetic acid and NaCl. The crystals were dissolved in 0·1 *N*. NaOH. An appropriate volume of this solution was added to a buffer containing 40 m*M* of each of glycine, sodium acetate, disodium orthophosphate, sodium barbiturate, sodium citrate, sodium lactate, and boric acid. Preliminary tests had shown that this buffer has a good capacity over the pH range from 3 to 10. The microburette was filled with $N \cdot H_2SO_4$. The sample cell contained 1·60 ml deuteroheminbuffer solution, the reference cell was filled with freshly boiled distilled water.

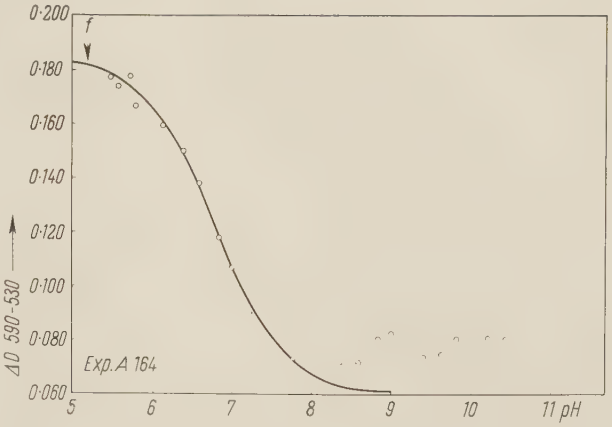


Fig. 2*b*.—The result of a typical spectrophotometric microtitration. Abscissa: pH. Ordinate: The difference of the extinction readings at a maximum (590 mμ) and a minimum (530 mμ) of the difference spectra of Fig. 2*A*. At *f*, the hemin begins to precipitate.

0 values of pH and ΔE actually read (corr. for dilution).

—— theoretical dissociation curve for pK 6·80 and n = 1.

The points at pH values of >8 suggest a further pH dependent reaction.

Figure 2*B* shows the result of such a titration. The pK value was found to be 6·8 ± 0·05 at a high ionic strength.

pK values reported for the replacement of the hydroxyl group associated with the hemin iron by a water molecule

Hemin	pK	Wavelength	Authors references
Protohemin IX	7·6	?	Shack and Clark[2]
Mesohemin IX	7·0	607 mμ	Cowgill a. Clark[3]
Deutero-hemin IX	6·8	590 530 mμ	this paper
Coprohemin I	7·44	600 mμ	Clark a. Perkins[4]

The Table lists the results of similar experiments with other hemins, published by Clark *et al.* They added aliquots of alkaline hemin solutions to buffers of known pH and measured the extinction of the resulting solution at the wavelengths indicated.

A. C. Maehly* and V. Legallais

The Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia (Pa), December 12, 1958.

Zusammenfassung

Ein Apparat zur spektrophotometrischen Titration kleiner Substanzmengen wird beschrieben. Er erlaubt die Messung von pH und optischer Dichte in einem Arbeitsgang und ist so konstruiert, dass er in einen gewöhnlichen Beckmann-Spektrophotometer hineinpasst. Als Beispiel wird die Titration von Deuterohämin beschrieben. Der erhaltene p*K*-Wert ist 6,8 ± 0,05.

[2] J. Shack and W. M. Clark, J. biol. Chem. *171*, 143 (1947).

[3] R. W. Cowgill and W. M. Clark, J. biol. Chem. *198*, 33 (1952).

[4] W. M. Clark and M. E. Perkins, J. biol. Chem. *135*, 643 (1940).

* Present address: Statens Rättskemiska Laboratorium, Stockholm (Sweden).

Der Einfluss von Desoxyribonuklease (DNase) auf die Grösse des penicillin-(Pc)resistenten Anteils einer Population von *M. pyogenes* var. *aureus* Sg 511

Die Induktion einer Pc-Resistenz oder anderer genetischer Faktoren durch eine dem Suspensionsmedium von Keimen zugesetzte DNS wird bekanntlich durch DNase aufgehoben. Nicht beschrieben wurde aber der mögliche Einfluss von DNase als solcher auf den pc-resistenten Anteil einer Keimpopulation. Untersucht wurde deswegen der Einfluss von kristalliner DNase (Dr. Schuchardt, München und C. Roth, Karlsruhe) auf die Grösse des pc-resistenten Anteils von *Staph. aureus* Sg 511.

Der Teststamm hatte nach dem üblichen Dilutionstest eine mittlere Sensibilität von 0,04 E Pc/ml. Zur Lösung von DNase bzw. zur Suspension der von einer 16 h alten Agarkultur stammenden und zweimal gewaschenen Testkeime diente eine 0,01 *M*-Lösung von $MgCl_2 \cdot 7\,H_2O$. Gleiche Volumina der Keimsuspension (mikroskopisch eingestellt auf 10^{10} Zellen je ml) und der Lösung von kristalliner DNase (400 µg/ml) wurden gemischt und 20 min im Wasserbad bei 37°C erwärmt. Zur Bestimmung der «Lebendkeimzahl» (Koloniezahl) wurden davon dann 10 Petrischalen (Nähragar) mit je 0,3 ml nach Vorverdünnung der Keimsuspension auf $0{,}2 \cdot 10^{-6}$ beschickt. Zur Bestimmung des resistenten Anteils (Koloniezahl) wurden weitere 10 Platten mit pc-haltigem Nähragar mit je 0,3 ml dieser Keimsuspension ohne Vorverdünnung beimpft.

Versuch-Nr.	Ansatz	Koloniezahl je ml Gesamt × 10^6 ± %	Koloniezahl je ml Re ± %	Re/10^9 Gesamt ± %	Vergleich von Re/10^9 Gesamt bei Kontrolle und Versuch t	P
1	DNase aktiv	403,3 ± 36,8	759,7 ± 18,3	1883,6 ± 18,3	5,1492	$<0{,}001$
	DNase inaktiv	458,3 ± 38,2	1236,3 ± 13,4	2697,6 ± 13,4		
2	DNase aktiv	421,7 ± 20,9	740,3 ± 14,7	1757,0 ± 14,7	3,1247	$<0{,}02$ ($>0{,}01$)
	DNase inaktiv	531,7 ± 17,1	1212,3 ± 20,2	2280,2 ± 20,2		

Die Konzentration des verwandten Na-Salzes von Pc-G wurde zur guten statistischen Erfassung so eingestellt, dass die Zahl der je Platte gewachsenen resistenten Kolonien mindestens 150 betrug, das heisst bei Versuch 1 = 0,06 E Pc/ml und bei Versuch 2 = 0,04 E Pc/ml. Als Kontrolle diente derselbe Ansatz mit der gleichen Keimsuspension, wobei lediglich die D-Nase-Lösung vorher 60 min auf Kochtemperatur gehalten worden war. Die Auszählung der auf Agar ohne und mit Pc-Zusatz gewachsenen Kolonien erfolgte nach 24 bzw. 48 h Bebrütung bei 37°C. Die Zahl der resistenten Kolonien einer jeden Platte (Re) wurde auf die mittlere Gesamtzahl der Kolonien bezogen und danach die Zahl der Re je 10^9 insgesamt gewachsenen Kolonien errechnet. Mit Students t-Test wurden die so bei Versuch und Kontrolle ermittelten Werte unter Berücksichtigung der Streuung statistisch verglichen.

Aus der Aufstellung ist zu entnehmen, dass durch Zugabe von kristalliner DNase der pc-resistente Anteil der Keimpopulation in statistisch signifikanter Weise kleiner wird, wenn man als Kriterium der Signifikanz eine Wahrscheinlichkeit von 2% für die Zufallsbedingtheit der Differenz annimmt.

G. Gillissen

Hygiene-Institut der Universität Mainz, 5. Januar 1959.

Summary

The number of penicillin-resistant organisms among a certain population of *Staph. aureus* can be reduced by treatment with crystalline desoxyribonuclease. These results are statistically significant.

INFORMATIONES

Congressus

GERMANY

Geochemical Symposium

Göttingen, August 21st to 24th, 1959

The Commission on Geochemistry of the International Union of Pure and Applied Chemistry will be holding a Symposium on:

(1) Stable Isotopes in Geochemistry;
(2) Long-living Natural Isotopes;
(3) Geochemistry of the Halogenes;
(4) Bio-Geochemistry

in Göttingen (Germany) on August 21st and 22nd, 1959, followed by Excursions on August 23rd and 24th, 1959.

Details may be obtained from Professor Dr. Correns, Sedimentpetrographisches Institut, Göttingen, Lotzestrasse 13.

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

Attività enolasica del plasma e del fegato nell'epatite sperimentale da virus MHV-3 Craig

Di F. de Ritis, M. Coltorti e G. Giusti*

Nostre precedenti ricerche hanno dimostrato che nell'epatite sperimentale da virus MHV-3 Craig si verifica il passaggio in circolo dai focolai di necrosi epatica, di numerosi enzimi, normalmente presenti in scarsa misura od assenti nel plasma: transaminasi aspartico-chetoglutarico ed alanina-chetoglutarico, fosfoglicomutasi, fumarasi, alcool-deidrogenasi, glutammico-deidrogenasi, fosforilasi [1–4].

L'estensione delle ricerche sulla «sindrome enzimoplasmatica» dell'infezione sperimentale da virus MHV-3 era giustificata dall'interesse di stabilire una comparazione con le variazioni enzimatiche plasmatiche osservate nell'epatite virale umana, alla cui conoscenza sono stati apportati molteplici contributi da noi e da altri ricercatori[5–17], in considerazione delle notevoli analogie patogenetiche ed anatomo-patologiche fra queste due malattie epatonecrotiche.

Le precedenti osservazioni dell'aumento nel plasma di diverse attività della glicolisi in condizioni di necrosi epatica virale o tossica (aldolasi, fosfoesosoisomerasi, fosfoglicomutasi[8,9,13–16,18–20]), ci ha fatto ritenere opportuno prendere in considerazione il comportamento dell'attività enolasica.

Alcuni dei fattori capaci di interferire nel determinismo del passaggio in circolo di enzimi dalla cellule lese, consistono presumibilmente nelle caratteristiche di solubilità, nelle dimensioni molecolari, nella localizzazione nelle varie strutture cellulari. Gli enzimi del ciclo di Embden-Meyerhof sono solubili: essi sono stati tutti studiati originariamente in estratti acquosi di tessuti e sono stati riscontrati pressoché interamente nella frazione solubile del citoplasma (Schneider[21]).

L'enolasi, com'è noto, catalizza la reazione che conduce alla conversione reversibile dell'acido 2-fosfoglicerico in acido fosfoenolpiruvico: D-2-PGA $\rightleftharpoons$ PEP + H_2O.

Contemporaneamente allo studio dell'attività enolasica nel plasma, abbiamo ritenuto opportuno indagare circa il comportamento della stessa attività enzimatica a livello del tessuto epatico, dal quale era logico ammettere che derivasse la quota di enzima responsabile dell'aumento dell'attività enzimatica plasmatica.

Inoltre, allo scopo di accertarci sè le variazioni di tale attività a livello del plasma nel corso della infezione dipendessero effettivamente dalla cessione e dal passaggio in circolo di molecole enzimatiche, e non di effettori o fattori coenzimatici capaci di attivare una eventuale quota di enzima presente nel plasma in forma inattiva, abbiamo condotto esperienze in cui l'attività enolasica del plasma di animali normali veniva saggiata anche in presenza di estratto bollito di fegato, di per sè enzimaticamente inattivo.

Materiali e metodi. Topi bianchi del peso medio di 20 g, tenuti a dieta di Vogt e Möller adeguatamente supplementata, sono stati infettati per via intraperitoneale con 100 LD_{50} di virus MHV-3 Craig[22].

Con tale modalità si determina una infezione sicuramente letale a rapida evoluzione, con morte degli animali tra la 72° e la 120° h. Gruppi di 3–4 animali sono stati sacrificati dopo 24, 48, 72, 96 h dall'infezione. Sui campioni di plasma ottenuti dai rispettivi *pools* di sangue raccolti in provette eparinizzate e sul sopranatante di omogenati di tessuto epatico al 2% in H_2O, eseguiti in apparecchi di Potter-Elvehjem di vetro a temperatura di ghiaccio fondente e quindi centrifugati per 5 min a 2000 r.p.m., l'attività enolasica è stata saggiata secondo la seguente metodica.

Puffer di glicocolla-NaOH 0,1 mol a pH 7,4 . . . ml 1,00
Acido D-2-fosfoglicerico 0,01 mol[23] . . . . . ml 1,00

* Istituto di patologia speciale medica e metodologia clinica dell'università di Napoli.

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[6] F. De Ritis, G. Giusti e M. Coltorti, Boll. Soc. Ital. Biol. Sperim. *32*, 386 (1956); Exper. *13*, 81 (1957); Minerva Med. *48*, 1598 (1957).
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[8] F. M. Bruns e W. Puls, Klin. Wschr. *32*, 656 (1954).
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[21] W. C. Schneider, 3° Congr. Internaz. di Biochimica, Bruxelles 1955, Atti, p. 143.
[22] Gentilmente fornito dal Dr. A. W. Gledhill, del National Institute for Medical Research, London.
[23] Acido *d* (+)-2-fosfoglicerico, sale di bario (Boehringer), trasformato in sale di sodio, neutralizzato e conservato a —15° fino al momento dell'uso.

$MgSO_4$ 0,016 mol . . . . . ml 0,50
Plasma . . . . . ml 0,20
oppure omogenato di fegato . . . . . ml 0,10
H_2O a volume finale di . . . . . ml 3,00

In alcuni saggi dell'attività enolasica plasmatica, alla miscela di incubazione veniva aggiunto 0,1 ml del sopranatante di un omogenato di fegato di topo al 15%, bollito per 45 sec e centrifugato per 10 min a 2000 r.p.m.

Tabella I

Attività enolasica nel plasma: µmoli di acido 2-fosfoglicerico convertito in acido fosfoenolpiruvico da 1 ml di plasma in 15 min a 20°C.

Ore dalla inoculazione del virus	N° di determinazioni eseguite	Attività enzimatica (media dei valori)	P
0	7	2,83	–
24	4	2,66	$> 0{,}05$
48	4	4,99	$< 0{,}05$
72	4	11,39	$< 0{,}001$
96	4	11,59	$< 0{,}001$

Incubazione a 20°C, 15 min per l'attività plasmatica, 20 min per quella epatica. Per le prove eseguite sul plasma si è proceduto alla deproteinizzazione con 2,00 ml di acido metafosforico al 15% e, dopo 10 min di attesa a temperatura ambiente, alla filtrazione attraverso filtri Schleicher-Schüll n. 602. Quindi a 2,00 ml del filtrato limpido si aggiungevano 1,00 ml di NaOH approssimativamente 1,1 N, il cui titolo era aggiustato in modo da portare il pH finale a 7,4. Si eseguivano quindi le letture allo spettrofotometro Beckman mod. DU, λ 240 mµ, con vaschette di quarzo dello spessore di 1 cm. Ai valori di estinzione delle prove enzimatiche erano sottratti i valori di prove identiche deproteinizzate al tempo 0. In sostanza s'è adottato il test ottico di Warburg e Christian seguendo complessivamente la descrizione di Bücher[24], cui è stato necessario aggiungere la deproteinizzazione a causa dell'elevato assorbimento di luce da parte del plasma. Per i saggi di attività enolasica epatica, si è invece proceduto alla lettura spettrofotometrica senza deproteinizzazione nè ulteriore diluizione della miscela di reazione.

Tabella II

Attività enolasica del tessuto epatico: µmoli di acido 2-fosfoglicerico trasformato in acido fosfoenolpiruvico da 10 mg di tessuto in 20 min a 20°C.

Ore dalla inoculazione del virus	N° di determinazioni eseguite	Attività enzimatica (media dei valori)	P
0	10	2,17	–
24	10	2,03	$> 0{,}05$
48	10	2,09	$> 0{,}05$
72	10	1,92	$> 0{,}05$
96	10	1,22	$< 0{,}01$

Risultati. I risultati delle nostre esperienze sono riportati nelle Tabelle. È stata saggiata la validità statistica delle differenze tra le medie dei valori degli animali controllo e di quelli nelle varie fasi dell'infezione, mediante l'indice *P* di Fisher[25].

Aggiungiamo inoltre che l'estratto di fegato bollito non ha provocato incremento dell'attività enolasica plasmatica dei topi normali e di quelli infettati.

Commento e conclusioni. L'attività enolasica nel fegato non subisce variazioni significative fino alla 72° ora dell'infezione e solo nella fase più tardiva (96° h) presenta una diminuzione notevole e statisticamente significativa. L'attività enolasica del plasma comincia invece ad aumentare in fasi della malattia nettamente precedenti quella in cui si verifica la sua caduta nel parenchima epatico. Quest'ultima, in effetti, compare solo in coincidenza del periodo terminale della malattia, caratterizzato da lesioni necrotico-degenerative praticamente estese a tutto il parenchima, ed è quindi da considerare un'espressione aspecifica del danno epatocellulare grave e diffuso.

Per l'interpretazione del comportamento dell'attività enolasica epatica nel corso dell'epatite da virus MHV-3, riteniamo opportuno tener presente quanto è stato da noi osservato e discusso a proposito dell'attività fosfoglicomutasica[2,4], la quale subisce nel fegato un incremento contemporaneo all'aumento dei livelli plasmatici, da noi interpretato come probabile espressione di resintesi dell'enzima, tanto intensa da superare la perdita derivante da passaggio di una notevole quantità di esso in circolo. La mancata diminuzione dell'attività enolasica epatica nelle fasi in cui questa è già nettamente aumentata nel plasma per cessione dell'enzima dagli epatociti lesi dal processo moltiplicativo virale, si può presumere dipenda da una sintesi *ex novo* di molecole enzimatiche, di entità tale da impedire la deflessione dell'attività enzimatica epatica, ma non tale da condurre, come nel caso della fosfoglicomutasi, ad un aumento del suo contenuto. Se questa ipotesi corrisponde ad una giusta interpretazione dei fatti osservati, non vi sarebbero differenze, sul piano concettuale, nel comportamento a livello del fegato delle due attività enzimatiche, poiché, a parte le differenze d'ordine quantitativo, esso in entrambi i casi sarebbe la conseguenza di analoghi processi di neosintesi enzimatica, correlata ai fenomeni di precoce rigenerazione epatica, qual'è nota avvenire nel corso dell'epatite virale e delle epatopatie necrotiche in genere.

La mancata attivazione dell'enolasi già presente normalmente nel plasma di topo, da parte di estratto di fegato reso enzimaticamente inattivo, dimostra che gli aumenti osservati nel corso della necrosi non sono dovuti ad attivazione di molecole di enzima già presenti nel plasma in forma inattiva, bensì a passaggio in circolo di una quota notevole di enzima completo.

Summary

In experimental hepatitis from MHV-3 Craig strain, the enolase activity of plasma increases significatively from 48th h of the infection, reaching the highest values at 72-96th h.

The same activity in the liver decreases only after 72th h, while in the precocious stages of the infection it is unmodified.

The physiopathological significance of the enzymatic modification in the plasma is related to the liver necrosis (enzymo-plasmatic syndrome of virus hepatitis).

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COGITATIONES

Neue Extremalprobleme über konvexe Rotationskörper

Von H. Bieri*

Die konvexen Rotationskörper, Träger der drei Hauptmasszahlen V, F, M und ausgestattet mit einer ganzen Serie von Nebenmasszahlen, geben Anlass zu vielerlei Extremalproblemen. Manche davon sind gelöst, aber vieles bleibt noch zu tun[1].

Die Beschäftigung mit diesen Problemen ist an sich reizvoll. Wichtiger noch ist indessen, dass sie im Zusammenhang mit einem Hauptproblem über konvexe Rotationskörper stehen, ja gewissermassen als unerlässliche Durchgangsstation auf dem Wege zur Lösung des Hauptproblems angesehen werden können. In der vorliegenden Arbeit werden zwei Sätze über konvexe Rotationskörper mit festem Äquatorradius $r = 1$, fester Länge l und festem Volumen V bewiesen[2]. Die Nebenbedingungen bewirken, dass *nichttriviale Maxima* von F und M auftreten. Zwecks Entlastung des Textes sei vorweggenommen, dass die Extremalkörper der Teilklasse I angehören müssen (Äquatorradius am Rand).

Für die Hauptmasszahlen der Kegelstümpfe (im weitern Sinne) erhält man:

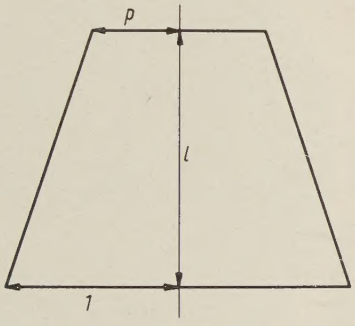


Abb. 1

$$\begin{aligned} \overline{V} &= \frac{\pi l}{3}(1+p+p^2) \\ \overline{F} &= \pi\left[1+p^2+(1+p)\sqrt{(1-p)^2+l^2}\right] \\ \overline{M} &= \pi\left[l+\pi-(1-p)\operatorname{arctg}\left(\frac{l}{1-p}\right)\right] \end{aligned} \tag{1}$$

Nach den Substitutionen

$$\begin{aligned} &\overline{V} = \frac{\pi}{3}\cdot V;\ \overline{F} = \pi\cdot F;\ \overline{M} = \pi\cdot M\,. \\ &1-p = l\cdot \mathfrak{Sin}\,\varphi;\ 0 \leqslant \mathfrak{Sin}\,\varphi \leqslant {}^1/l \end{aligned} \tag{2}$$

gehen die Formeln (1) über in

$$\begin{aligned} V &= l(3-3\,l\cdot \mathfrak{Sin}\,\varphi + l^2\cdot \mathfrak{Sin}^2\varphi) \\ F &= 2+2\,l\cdot e^{-\varphi} - l^2\cdot \mathfrak{Sin}\,\varphi\cdot e^{-\varphi} \\ M &= l+\pi-l\cdot \mathfrak{Sin}\,\varphi\cdot \operatorname{arctg}\left(\frac{1}{\mathfrak{Sin}\,\varphi}\right) \end{aligned} \tag{3}$$

A. *Das (F, V)-Problem*

Es gilt der

Satz I: Unter allen konvexen Rotationskörpern von festem Aquatorradius $r=1$, fester Länge $l \geqslant (1+\sqrt{13})/2$ und festem Volumen V besitzen Kegelstümpfe im weitern Sinne und nur diese Körper grösste Oberfläche. F.

Beweis: Man erhält:

$$\dot{F} = -l\cdot e^{-2\varphi}(l+2\,e^{\varphi});\ \ \dot{V} = l^2\,\mathfrak{Cos}\,\varphi\,(2\,l\cdot \mathfrak{Sin}\,\varphi - 3) < 0$$

$$\frac{dF}{dV} = \frac{2}{l}\cdot\frac{(l+2\,e^{\varphi})}{(l+3\,e^{\varphi}+3\,e^{3\varphi}-l\,e^{4\varphi})}$$

$$\frac{d^2F}{dV^2} = \frac{2}{l}\cdot\frac{1}{(l+3\,e^{\varphi}+3\,e^{3\varphi}-l\,e^{4\varphi})^2}\cdot\frac{1}{\dot{V}}\cdot e^{\varphi}\cdot\Phi(\varphi)\ ; \tag{4}$$

$$\Phi(\varphi) \equiv [6\,l\cdot e^{4\varphi}+(4\,l^2-12)\,e^{3\varphi}-9\,l\cdot e^{2\varphi}-l]$$

Vermittelst der Substitution

$$\begin{aligned} &4\,l^2-12 = 4\,\lambda\cdot l\ ;\ \ 0 \leqslant l < \infty \\ &l = \frac{\lambda+\sqrt{\lambda^2+12}}{2}\ ;\ -\infty < \lambda < +\infty \end{aligned} \tag{5}$$

erhält man die bequemere Form

$$\Phi(\varphi) \equiv l[6\,e^{4\varphi}+4\,\lambda\cdot e^{3\varphi}-9\,e^{2\varphi}-1]\ , \tag{6}$$

aus welcher unmittelbar gefolgert werden kann, dass für $\lambda \geqslant 1$, also für

$$l \geqslant \frac{1+\sqrt{13}}{2}$$

gemäss (4) die *Kegelstumpfkurve von unten konkav ist.*

Nun wenden wir uns den Doppelkegelstümpfen I_2 zu. Ihre Masszahlen berechnen sich zu

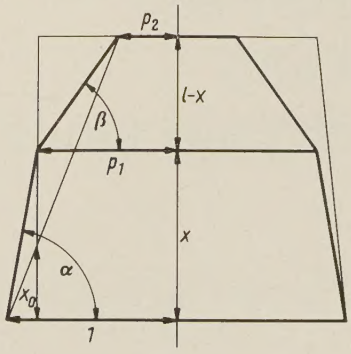


Abb. 2

$$\begin{aligned} F &= 1+p_2^2+(1+p_1)\sqrt{(1-p_1)^2+x^2}+(p_1+p_2)\sqrt{(p_1-p_2)^2+(l-x)^2} \\ V &= x\,(1+p_1-p_1p_2-p_2^2)+l\,(p_1^2+p_1p_2+p_2^2);\ x_0 \leqslant x \leqslant l \end{aligned} \tag{7}$$

* Bern.

[1] H. Hadwiger, *Vorlesungen über Inhalt, Oberfläche und Isoperimetrie* (Springer-Verlag 1957). – H. Bieri, Exper. *14*, 113 (1958).

[2] Die Beschränkung auf $r = 1$ ist unwesentlich.

Es folgt:

$$F_x = [(1+p_1)\sin\alpha - (p_1+p_2)\sin\beta];\quad V_x = \text{konstant.} \tag{8}$$

Mit wachsendem x nimmt α monoton zu, β monoton ab und daher dF/dv monoton zu. Dies aber hat zur Folge, dass die betrachtete Kurve im ganzen zulässigen Intervall von unten konvex ist und, wie Abb. 3 lehrt, kein Doppelkegelstumpf aus I_2 extremal sein kann.

Die angewendete Schlussweise ist, wie man sich leicht überzeugt, durchgreifend, und man sieht ein, dass kein Rotationskörper aus $I_n (n = 3, 4\ldots)$ extremal sein kann.

Infolgedessen ist der Kegelstumpf in der ganzen Klasse I und nach den einleitenden Ausführungen mit der genannten Einschränkung überhaupt extremal.

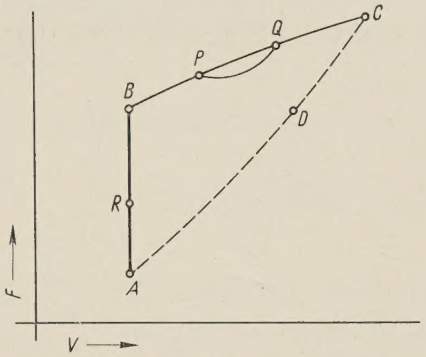


Abb. 3

A Symmetrischer Doppelkegel
B Kegel
PQ Kegelstümpfe
C Zylinder
D Parallelkörper der Strecke
R Unsymmetrischer Doppelkegel

B. *Das (M, V)-Problem*

Es gilt der

Satz II: Unter allen konvexen Rotationskörpern vom festen Äquatorradius $r = 1$, fester Länge $l \geqslant 6/\pi$ und festem Volumen V besitzen Kegelstümpfe im weitern Sinne und nur diese Körper grösstes Integral der mittlern Krümmung M.

Beweis: Man erhält:

$$\dot{M} = -l \cdot \mathfrak{Cos}\,\varphi \cdot \operatorname{arctg}\left(\frac{1}{\mathfrak{Sin}\,\varphi}\right) + l \cdot \mathfrak{Tg}\,\varphi$$

$$\frac{dM}{dV} = \frac{1}{l} \cdot \frac{[(1+\mathfrak{Sin}^2\varphi)\operatorname{arctg}\left(\frac{1}{\mathfrak{Sin}\,\varphi}\right) - \mathfrak{Sin}\,\varphi]}{(1+\mathfrak{Sin}^2\varphi)\,(3-2\,l\cdot\mathfrak{Sin}\,\varphi)} \tag{9}$$

Mit Hilfe der Substitution $\mathfrak{Sin}\,\varphi = 1/z\ ;\ \infty > z \geqslant l$ folgt weiter

$$\frac{dM}{dV} = \frac{1}{l} \cdot \frac{z\left[\operatorname{arctg} z - \frac{z}{1+z^2}\right]}{(3\,z-2\,l)} \quad \text{und sodann}$$

$$\frac{d^2M}{dV^2} = \frac{1}{l} \cdot \frac{\left\{(3\,z-2\,l)\left[\frac{z}{1+z^2} + \operatorname{arctg} z - \frac{2\,z}{(1+z^2)^2}\right] - 3\left[z\cdot\operatorname{arctg} z - \frac{z^2}{1+z^2}\right]\right\}}{(3\,z-2\,l)^2 \cdot V_z} \tag{10}$$

Das Vorzeichen von d^2M/dV^2 wird massgebend bestimmt durch den indefiniten Ausdruck

$$\Phi(z) \equiv \frac{3z^4 - lz^3 + lz}{(1+z^2)^2} - l \cdot \operatorname{arctg}(z) \tag{11}$$

Nun ist

$$\Phi(0) = 0;\ \Phi(\infty) = 3 - l\cdot\pi/2, \text{ also } \Phi(\infty) < 0 \text{ für } l > 6/\pi;$$
$$\Phi'(z) = \frac{-4\,z^2(2\,l-3\,z)}{(1+z^2)^3}, \text{ also } \Phi'(z) > 0 \text{ für } l \leqslant z < \infty. \tag{12}$$

Aus (12) folgt aber sofort, dass die Kegelstumpfkurve für $l \geqslant 6/\pi$ beständig *von unten konkav* ist.

Gemäss Abb. 2 erhält man:

$$M = l + \pi - \left[(1-p_1)\operatorname{arctg}\left(\frac{x}{1-p_1}\right) + (p_1-p_2)\operatorname{arctg}\left(\frac{l-x}{p_1-p_2}\right)\right]$$

$$M_x = \sin^2\alpha - \sin^2\beta\,.$$

Die betrachtete Kurve ist also auch diesmal *von unten konvex*, und somit kann kein Doppelkegelstumpf extremal sein. Analog zum Satz I gelangt man nunmehr zum Resultat, *dass der Kegelstumpf in der vollen Klasse der mit den vorliegenden Nebenbedingungen verträglichen konvexen Rotationskörper grösstes M besitzt*[3].

Wegen $(1+\sqrt{3})/2 > 6/\pi$ besitzt der Kegelstumpf für $\infty > l \geqslant 6/\pi$ *simultan beide Extremaleigenschaften*, was für das Hauptproblem der konvexen Rotationskörper von Bedeutung ist[4–6].

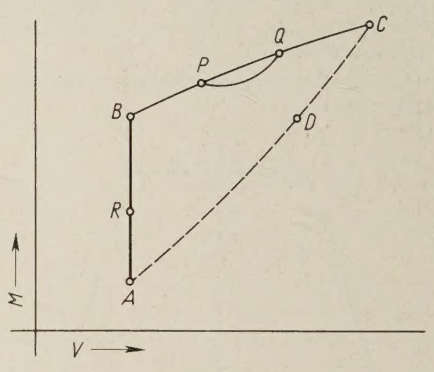


Abb. 4

A Symmetrischer Doppelkegel
B Kegel
PQ Kegelstümpfe
C Zylinder
D Parallelkörper der Strecke
R Unsymmetrischer Doppelkegel

Summary

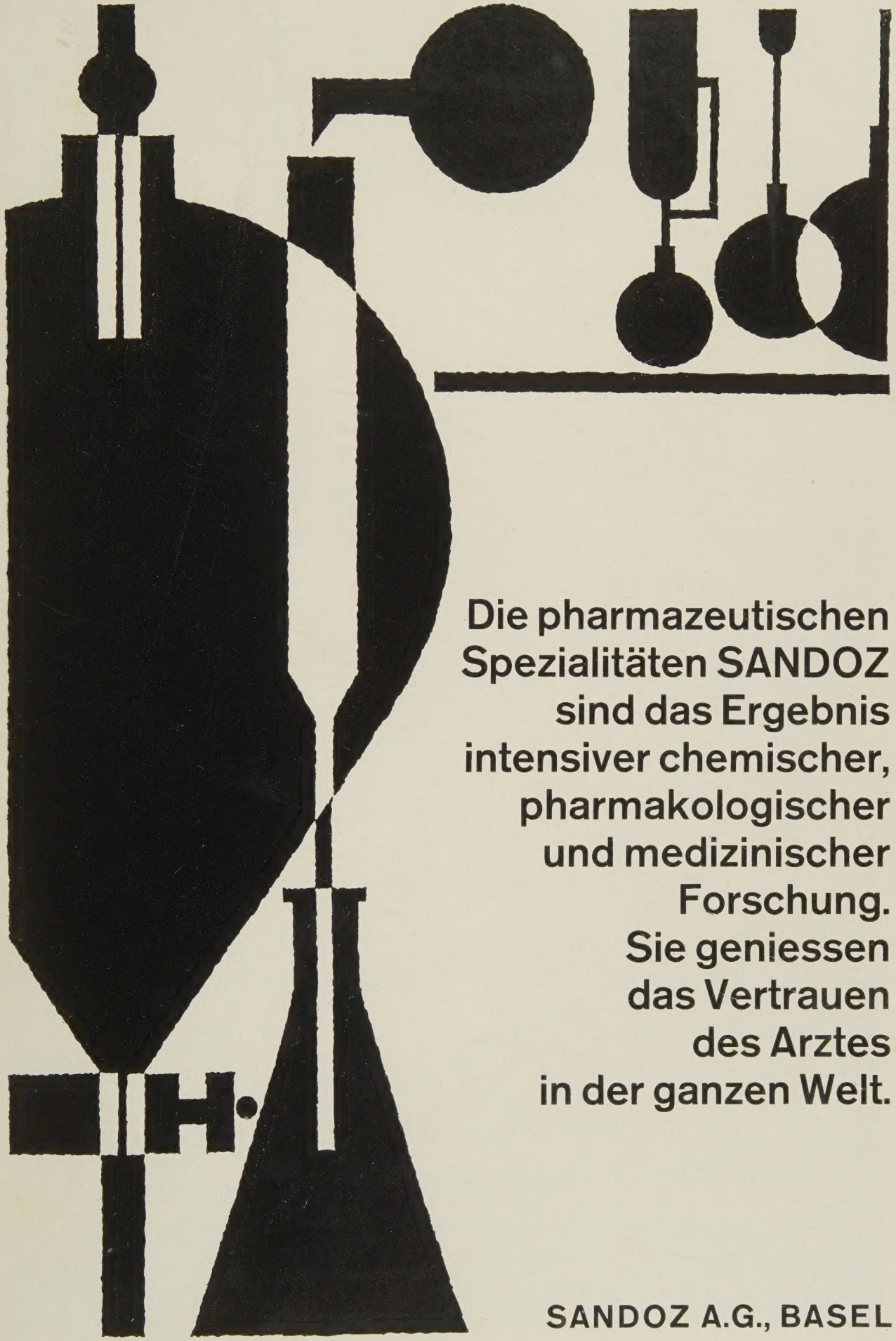
It is shown in this note that every sufficiently elongated conic frustum assumes, if radius of equator r, length l and volume V of a convex body of revolution are given, largest surface F as well as largest integral of mean curvature M. The conic frustum is the only extremal body. For small l, the situation is intricated and has not yet been clarified.

[3] Vgl. Abb. 4.

[4] Das günstige Intervall für l kann sicher in beiden Problemen noch etwas ausgedehnt werden, so im (F, V)-Problem bis in die nächste rechtsseitige Umgebung von $l = 1$, und zwar für alle zulässigen V, bis $l = {}^2/\sqrt{5}$ aber nur noch für kleine zulässige V.

[5] In beiden Problemen besitzt die *Kegelstumpfkurve* im zulässigen Intervall genau eine *Wendestelle*. Dieser Umstand kann bequem zur Gewinnung einer unscharfen Schranke für F bzw. M ausgenützt werden.

[6] Im (F, V)-Problem besitzen nach meinen Wahrnehmungen für $l < {}^2/\sqrt{5}$ gewisse *Doppelkegelstümpfe* die Extremaleigenschaft, allerdings nur für kleines V. Für grosses Volumen ist dagegen der *Zylinderkegel* im Intervall $0 \leqslant l < 1 + \varepsilon$ (ε klein) jedenfalls extremal. Die vorliegenden Verhältnisse sind noch nicht restlos abgeklärt.



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